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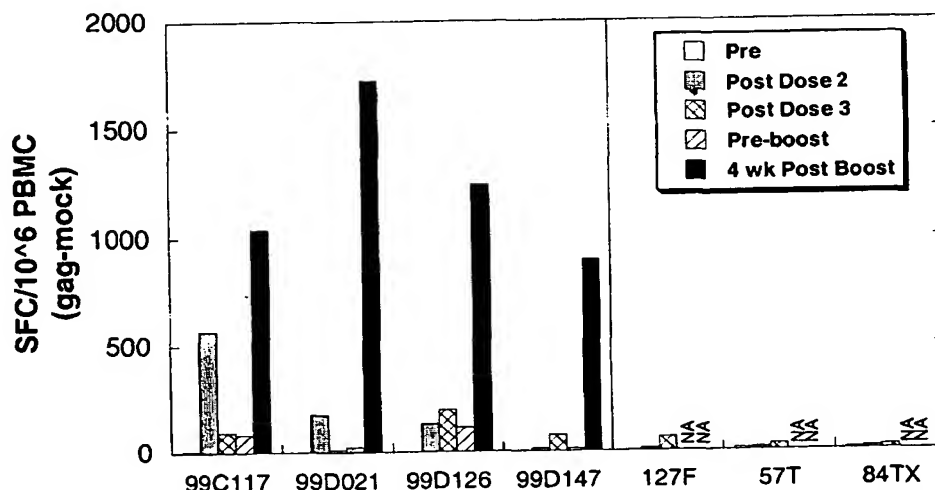
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- (71) Applicant (for all designated States except US): **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **EMINI, Emilio, A.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SHIVER, John, W.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHASTAIN, Michael** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
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(54) Title: **METHOD OF INDUCING AN ENHANCED IMMUNE RESPONSE AGAINST HIV**



(57) Abstract: An efficient means of inducing an immune response against human immunodeficiency virus ("HIV") utilizing specific prime-boost regimes is disclosed. The specific prime-boost regimes employ a heterologous prime-boost protocol wherein recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding a common HIV antigen are administered in that order. Vaccines administered into living vertebrate tissue in accordance with the disclosed regimes, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 antigen (e.g., Gag), inducing a cellular immune response which specifically recognizes HIV-1. It is believed that the disclosed prime/boost regime will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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TITLE OF THE INVENTION

METHOD OF INDUCING AN ENHANCED IMMUNE RESPONSE AGAINST HIV

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5           The present application claims priority to provisional applications U.S. Serial Nos. 60/363,870 and 60/392,581, filed March 13, 2002 and June 27, 2002, respectively, hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

- 10           Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

15   FIELD OF THE INVENTION

- The present invention relates to an enhanced means for inducing an immune response against human immunodeficiency virus ("HIV") utilizing recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding an HIV antigen in a heterologous prime-boost administration in the order specified.
- 20   Applicants have found that the poxvirus administration in this scheme very effectively boosts the adenovirus-primed immune response against HIV. Viruses of use in the instant invention can be any adenovirus or poxvirus, provided that the specific virus utilized is capable of effecting expression of exogenous genetic material introduced into the viral sequence. It is, further, imperative that the virus be replication-
- 25   defective, host restricted, or modified such that the virus does not freely replicate within the cells of a treated mammalian host. Specific embodiments of the instant invention employ an adenovirus vehicle which is replication-defective and specifically devoid of E1 activity in the priming administration. Further specific embodiments of the instant invention employ modified vaccinia viruses (such as
- 30   Modified Vaccinia Virus Ankara ("MVA"), or NYVAC, a highly attenuated strain of vaccinia virus) in the boosting administration. Alternative embodiments employ, for instance, a poxvirus selected from the group consisting of canarypoxviruses (such as ALVAC), other fowlpoxviruses and cowpoxviruses. Applicants have found that administration of a recombinant adenoviral vehicle comprising exogenous genetic

material encoding an antigen (specifically, an HIV antigen) followed by subsequent administration of recombinant poxvirus comprising the antigen notably amplifies the response from the initial administration(s) over and above that observed when the antigen is delivered via the recombinant adenoviral or poxviruses independently for both priming and boosting administrations, hence, offering an enhanced immune response. The effective boosting of the adenovirus-primed immune response with poxvirus leads to a significantly enhanced immune response capable of specifically recognizing HIV which is particularly manifest in the cellular immune response. Based on the above findings, it is believed that the disclosed prime/boost regime will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

Effective treatment regimes for HIV-1 infected individuals have become available. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. For instance, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the



kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

Adenoviral vectors have been developed as live viral vectors for delivery and expression of various foreign antigens including HIV and have proven to be effective in eliciting a CTL response in treated individuals. Adenoviruses are non-enveloped viruses containing a linear double-stranded genome of about 36 kb. The vectors achieve high viral titres, have a broad cell tropism, and can infect nondividing cells. Adenoviral vectors are very efficient gene transfer vehicles and are frequently used in clinical gene therapy studies. In addition, adenovirus has formed the basis of many promising viral immunization protocols.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including

*env* or *gag*. Various treatment regimes based on these vectors were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

5 Replication-defective adenoviral vectors harboring deletions, for instance, in the E1 region constitute a safer alternative to their replicating counterparts. Recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging  
10 efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Vaccinia virus and other poxviruses (*e.g.*, avipoxviruses) have been disclosed as promising vaccine candidates for their demonstrated high-level expression of  
15 proteins and have been considered recently for the delivery and expression of HIV antigens. Poxviruses are large, enveloped viruses with double-stranded DNA that is covalently closed at the ends. These viruses possess a high insertion capacity for multiple foreign genes and obtain high level cytoplasmic expression of exogenous foreign genetic material. Their use as vaccines has been known since the early  
20 1980's; *see, e.g.*, Panicali *et al.*, 1983 *Proc. Natl. Acad. Sci. USA* 80:5364-5368. Live recombinant vaccines have been tested in clinical trials using recombinant vaccinia virus or canarypoxvirus for expression of the HIV-1 envelope, and the major Epstein-Barr virus membrane glycoprotein or the rabies virus glycoprotein for the induction of immune responses; *e.g.*, Paoletti, 1996 *Proc. Natl. Acad. Sci. USA* 93:11349-53; Gu *et al.*, 1995 *Dev. Biol. Stand.* 84:171-7; and Fries *et al.*, 1996 *Vaccine* 14:428-34.  
25

Administration protocols employing viral vaccine vectors to date have employed various prime-boost inoculation schemes. Two general schemes frequently used are: (1) wherein both priming and boosting of the mammalian host is accomplished using the same virus vehicle, and (2) wherein the priming and boosting  
30 is carried out utilizing different vehicles not necessarily limited to virus vehicles. Examples of the latter are, for instance, a scheme composed of a DNA prime and viral boost, and one composed of a viral prime and a viral boost wherein alternate virus are used. Recently, a prime-boost regime of the latter scheme employing a combination of two of the above viruses, adenovirus and poxvirus, in varying order (*i.e.*,

adenovirus-prime, poxvirus-boost; and poxvirus-prime, adenovirus-boost) was utilized to effect the delivery and expression of the CS gene of *Plasmodium berghei* (Ad-PbCS) to mice; Gilbert *et al.*, 2002 *Vaccine* 20:1039-45. This strategy was disclosed to be protective in mice against malaria; *see, e.g.*, Gilbert *et al.*, 2002  
5 *Vaccine* 20:1039-45.

It would be of great import in the battle against AIDS to develop a prophylactic- and/or therapeutic-based HIV vaccine strategy capable of generating a strong cellular immune response against HIV infection. The present invention addresses and meets these needs by disclosing a heterologous prime-boost HIV  
10 immunization regime based on the administration of recombinant adenoviral and poxvirus vectors comprising exogenous genetic material encoding a common HIV antigen. The specific prime-boost vaccination regime is one wherein an individual is primed with the recombinant adenoviral vector and then provided a boosting dose of the recombinant poxvirus vector. A vaccine protocol in accords with this description,  
15 as far as Applicants are aware, has not been demonstrated for HIV. This vaccine prime-boost regime may be administered to a host, such as a human.

#### SUMMARY OF THE INVENTION

The present invention relates to an enhanced method for generating an  
20 immune response against human immunodeficiency virus ("HIV"). The method is based on the heterologous prime-boost administration of recombinant adenoviral and poxvirus vectors comprising heterologous genetic material encoding an HIV antigen to effect a more pronounced immune response against HIV than that which can be obtained by either vector independently in a single modality prime-boost  
25 immunization scheme. A mammalian host is first administered a priming dose of adenovirus comprising a gene encoding the HIV antigen and, following some period of time, administered a boosting dose of poxvirus carrying the gene encoding the HIV antigen. There may be a predetermined minimum amount of time separating the administrations, which time essentially allows for an immunological rest. In  
30 particular embodiments, this rest is for a period of at least 4 months. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. Applicants have found that boosting of the adenovirus-primed response with poxvirus in this manner leads to a notably

amplified immune response to the HIV antigen. Thus the instant invention relates to the administration of adenovirus and poxvirus HIV vaccines in this manner.

Accordingly, the instant invention relates to a method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host comprising the steps of (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter (b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof.

The adenoviral and poxvirus vectors utilized in the immunization regimes of the present invention may comprise any replication-defective adenoviral vector and any replication-defective, replication-impaired or host-restricted poxvirus vector which is genetically stable through large scale production and purification of the virus. In other words, recombinant adenoviral and poxvirus vectors suitable for use in the methods of the instant invention can be any purified recombinant replication-defective, replication-impaired or host-restricted virus shown to be genetically stable through multiple passages in cell culture which remains so during large scale production and purification procedures. Such a recombinant virus vector and harvested virus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of an immunization regime which is based on the use of recombinant replication-defective adenovirus and poxvirus vectors of decreased virulence.

Poxviruses have been the subject of various genetic engineering efforts designed to reduce the virulence of the virus. For instance, efforts with vaccinia virus targeted the viral thymidine kinase, growth factor, hemagglutinin, 13.8 kD secreted protein and ribonucleotide reductase genes; *see Buller et al.*, 1985 *Nature* 317(6040):813-815; Buller *et al.*, 1988 *J. Virol.* 62(3):866-74; Flexner *et al.*, 1987 *Nature* 330(6145):259-62; Shida *et al.*, 1988 *J. Virol.* 62(12):4474-80; Kotwal *et al.*, 1989 *Virology*. 171(2):579-87; and Child *et al.*, 1990 *Virology* 174(2):625-9. Modified vaccinia viruses form the subject of, *inter alia*, U.S. Patent Nos. 5,185,146; 5,110,587; 4,722,848; 4,769,330; 5,110,587; and 4,603,112. Avipoxviruses also are

of interest as they possess a limited host range and, therefore, do not freely replicate in human cells. Recombinant avipoxviruses are the subject of, *inter alia*, U.S. Patent Nos. 5,505,941; 5,174,993; 5,942,235; 5,863,542; and 5,174,993. U.S. Patent No. 5,266,313 discloses a raccoon poxvirus-based vaccine for rabies virus. The poxvirus vector of choice is administered to boost the immune response activated by the prior administration of an adenovirus vehicle carrying an HIV transgene.

Adenoviral vectors of use in the instant invention are those that are at least partially deleted in E1 and devoid of E1 activity. Vectors in accordance with this description can be readily propagated in E1-complementing cell lines, such as PER.C6® cells.

The recombinant adenoviral and poxvirus vectors of use in the instant application comprise a gene encoding an HIV antigen. In specific embodiments, the gene encoding the HIV antigen or immunologically relevant modification thereof comprises codons optimized for expression in a mammalian host (*e.g.*, a human). In preferred embodiments, the adenoviral and/or poxvirus vectors comprise a gene expression cassette comprising (a) a nucleic acid encoding an HIV antigen (*e.g.*, an HIV protein) or biologically active and/or immunologically relevant portion/modification thereof; (b) a heterologous (non-native) or modified native promoter operatively linked to the nucleic acid of part a); and, (c) a transcription termination sequence; provided that any promoter utilized to drive expression of the nucleic acid included within the gene expression cassette for the recombinant poxvirus vector is either native to, or derived from, the poxvirus of interest or another poxvirus member. Naturally occurring, nonoverlapping, tandem early/late promoters of moderate strength have been described for vaccinia virus (*see, e.g.*, Cochran, *et al.*, 1985 *J. Virol.* 54:30-37; and Rosel *et al.*, 1986 *J. Virol.* 60:436-9) and have been used for gene expression.. An example of a modified native promoter is the synthetic early/late promoter of Example 2, previously described in Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-97. A heterologous promoter can be any promoter under the sun (modified or not) which is not native to, or derived from, the virus in which it will be used. Preferably, the gene expression cassette used within the recombinant poxvirus comprises (a) a nucleic acid encoding an HIV antigen (*e.g.*, an HIV protein) or biologically active and/or immunologically relevant portion/modification thereof; and (b) a heterologous promoter (from another poxvirus species) or a promoter which is native to or derived from the poxvirus of interest.

HIV antigens of use in the instant invention include the various HIV proteins, immunologically relevant modifications, and immunogenic portions thereof. The present invention, thus, encompasses the various forms of codon-optimized HIV-1 gag (including but by no means limited to p55 versions of codon-optimized full length ("FL") Gag and tPA-Gag fusion proteins), HIV-1 pol, HIV-1 nef, HIV env, fusions of the above constructs, and selected modifications of the above possessing immunological relevance. Examples of HIV-1 Gag, Pol, Env, and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

Recombinant viral vectors in accordance with the instant disclosure form an aspect of the instant invention. Other aspects of the instant invention are host cells comprising said adenoviral and/or pox virus vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral and/or pox virus vector into a host cell, and (b) harvesting the resultant vectors.

The present invention also relates to prime-boost regimes wherein the recombinant adenoviral and poxvirus vectors comprise various combinations of the above HIV antigens. Such HIV immunization regimes will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include viral vector-based multivalent vaccine compositions which provide for a divalent (*e.g.*, gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (*e.g.*, gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component. To this end, preferred vaccine compositions for use within the instant methods are adenovirus and poxvirus vectors comprising multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regime.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a recombinant viral vector comprising multiple open reading frames. For example, a trivalent vector may  
5 comprise a gag-pol-nef fusion, or possibly a "2+1" divalent vaccine comprising, for instance, a gag-pol fusion (*e.g.*, codon optimized p55 gag and inactivated optimized pol) within the same backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the  
10 open reading frames operatively linked by an internal ribosome entry sequence (IRES).

Administration of the recombinant adenoviral and poxvirus vectors via the disclosed heterologous means provides for improved cellular-mediated immune responses; responses that are more pronounced than that afforded by single modality  
15 regimes. An effect of the improved vaccine (adenoviral HIV prime and poxvirus HIV boost) should be a lower transmission rate to previously uninfected individuals (*i.e.*, prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (*i.e.*, therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. The administration, intracellular delivery and expression of  
20 the vaccine in this manner elicits a host CTL and Th response. The individual vaccinee or mammalian host (as referred to herein) can be a primate (both human and non-human) as well as any non-human mammal of commercial or domestic veterinary importance.

In light hereof, the present invention relates to methodology regarding  
25 administration of the adenoviral and poxvirus vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. Such treatment regimes may include a  
30 monovalent or multivalent composition, and/or various combined modality applications. Therefore, the present invention provides for methods of using the disclosed HIV vaccine administration scheme within the various parameters disclosed herein as well as any additional parameters known in the art which, upon introduction

into mammalian tissue, induces intracellular expression of the HIV antigen(s) and an effective immune response to the respective HIV antigen(s).

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given the recombinant adenovirus and poxvirus HIV vaccines in accordance with the disclosed heterologous prime-boost immunization regime.

As used throughout the specification and claims, the following definitions and abbreviations are used:

- "HAART" refers to -- highly active antiretroviral therapy --.
- "first generation" vectors are characterized as being replication-defective. They typically have a deleted or inactivated E1 gene region, and often have a deleted or inactivated E3 gene region as well.
- "AEX" refers to Anion Exchange chromatography.
- "QPA" refers to Quick PCR-based Potency Assay.
- "bps" refers to base pairs.
- "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.
- "PBMCs" refers to peripheral blood monocyte cells.
- "FL" refers to full length.
- "FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.
- "Ad5-Flgag" refers to an adenovirus serotype 5 replication-deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.
- "Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.
- "Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results in a protein having an N-terminal peptide extension, often referred to as a pro-sequence.
- "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and therefore not transcribed into mRNA or translated into protein.



"Immunologically relevant" or "biologically active," when used in the context of a viral protein, means that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual.

5 The same terms, when used in the context of a nucleotide sequence, means that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

10 "bGHpA" refers to a bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the tissue plasminogen activator leader sequence and an optimized HIV gag gene.

15 Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

20 "Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal.

25 "MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector which is deleted of E1, and contains adenoviral base pairs 1-450 and 3511-3523, with a human codon-optimized HIV-1 gag gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

30 "pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-

bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

5 "pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

10 "pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) 15 sequences from bp 1 to bp 450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation.

20 "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intron A) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique 25 *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from base pairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized 30 HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA".

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human

codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHPA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

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#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the HIV-1 gag adenovector "Ad5HIV-1gag". This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999, and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

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Figure 2 shows the nucleic acid sequence (SEQ ID NO: 1) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the transgene construct disclosed in PCT International Application No. PCT/US01/28861, filed September 14, 2001 in comparison with the original gag transgene. PCT International Application No. PCT/US01/28861 claims priority to U.S. Provisional Application Serial Nos. 60/233,180, 60/279,056, and 60/317,814, filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively; the above applications all of which are hereby incorporated by reference.

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Figure 4 shows the modifications made to the adenovector backbone of Ad5HIV-1gag in the generation of the vector disclosed in PCT International Application No. PCT/US01/28861 which is utilized in certain examples of the instant application.

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Figure 5 shows the levels of Gag-specific T cells in rhesus macaques immunized with (a) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag and a single booster shot with 10e9 vp MRKAd5 HIV-1 gag ("10e9 vp MRKAd5-10e9 vp MRKAd5"); (b) two priming doses of 10e9 pfu MVA HIV-1 gag and a single booster with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"); or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). The levels expressed as number of spot-forming cells (SFC) per million PBMC are the mock-corrected values for each animal prior to the start of the immunization regimen ("Pre"); 4 weeks after the first priming dose ("Post Dose 1"); 4 weeks after the second

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priming dose ("Post Dose 2"); just prior to the boost ("Pre-Boost"); 4 weeks after the boost ("4 wks Post-Boost"); and 8 weeks after the boost ("8 wks Post-Boost"). For #99D241, data at 4 weeks post boost were unavailable (NA) because of poor PBMC yields.

5        Figure 6 shows the Gag-specific T cell responses induced by two priming doses of  $10^7$  vp dose of MRKAd5 HIV-1 gag (week 0; week 4) followed by administration of  $10^7$  vp MVA HIV-1 gag at week 27. The levels provided are the mock-corrected levels for each animal prior to the start of the immunization regimen ("Pre"); 4 weeks after the first priming dose ("Post Dose 1"); 4 weeks after the second priming dose ("Post Dose 2"); just prior to the boost ("Pre-Boost"); 4 weeks after the boost ("4 wk Post-Boost"); and 8 weeks after the boost ("8 wk Post-Boost"). One will note a significant increase compared to the levels just prior to the boost. MVA-HIVgag elicited a large amplification of the priming response, with levels reaching as high as 1000 SFC/ $10^6$  PBMCs. Because the dose of MVA used as a booster shot induced weak or undetectable immune response in naïve animals (see Figure 5), the post-boost increases shown is largely attributed to the expansion of memory T cells instead of priming of new lymphocytes.

15        Figure 7 shows ELISPOT responses in BALB/c mice immunized with (1) one dose of  $5 \times 10^8$  vp Ad5 HIV-1 gag ("Ad5 prime-no boost"), (2) one dose of  $5 \times 10^8$  vp Ad5 HIV-1 gag followed by one dose of  $5 \times 10^6$  pfu vaccinia-gag ("Ad5 prime-Vacc Boost"), or (3) one dose of  $5 \times 10^6$  pfu vaccinia-gag ("Vacc prime-no boost"); Ad5-gag being the original gag vector discussed throughout the specification. The response in totally naïve animals was also assayed. Shown are the mock-corrected frequencies of T cells specific for a defined gag CD8+ epitope in BALB/c mice (AMQMLKETI). Ad5-primed immune responses (about 300 per million) were boosted significantly by administration of vaccinia-gag (to about 1400 per million).

20        Figure 8 shows a restriction map of the pMRKAd5HIV-1gag vector.

25        Figures 9A-1 to 9A-45 illustrate the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:2 [coding] and SEQ ID NO:3 [non-coding]).

30        Figure 10 shows the levels of Gag-specific antibodies in rhesus macaques immunized with (a) two priming doses of  $10^9$  vp of MRKAd5 HIV-1 gag and a single booster shot with  $10^9$  vp MRKAd5 HIV-1 gag ("10<sup>9</sup> vp MRKAd5-10<sup>9</sup> vp MRKAd5"), (b) two priming doses of  $10^9$  pfu MVA HIV-1 gag and a single booster

with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"), or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). Shown are the geometric mean titers for each cohort at the start of the immunization regimen ("Pre"), 4 weeks after the first priming dose ("Wk 4"), 4 weeks after the second priming dose ("Wk 8"), just prior to the boost ("Pre-Boost"), and 8 weeks after the boost ("Post-Boost").

Figure 11 shows the homologous recombination protocol utilized to recover pAd6E1-E3+ disclosed herein

Figure 12 shows the levels of Gag-specific T cells in rhesus macaques immunized with three doses of either MRKAd5-HIVgag or MRKAd6-HIVgag followed by a single booster shot with 10<sup>8</sup> pfu of ALVAC-HIVgag (see Table 4). Also shown are the responses in macaques given three (3) doses of 10<sup>9</sup> pfu ALVAC-HIVgag. The levels shown are the mock-corrected levels for each animal prior to the start of the immunization regimen ("Pre"), 4-8 wks after the second priming dose ("Post Dose 2"), 8 wks after the third vaccine dose ("Post Dose 3"), just prior to the boost ("Pre-Boost"), and 4 wks after the boost ("4 wk Post Boost"). For the 127F, 57T, and 84TX subjects, no vaccine (NA-not available) was given after the third ALVAC dose.

## DETAILED DESCRIPTION OF THE INVENTION

An enhanced means for generating an immune response against human immunodeficiency virus ("HIV") is described. The method is based on a heterologous prime-boost immunization scheme employing recombinant adenovirus and poxvirus vectors comprising exogenous genetic material encoding an HIV antigen (or antigens) of interest. A priming dose of the HIV antigen(s) is first delivered with a recombinant adenoviral vector. This dose effectively primes the immune response so that, upon subsequent identification of the antigen in the circulating immune system, the immune response is capable of immediately recognizing and responding to the antigen within the host. The priming dose(s) is then followed up with a boosting dose of a recombinant poxvirus vector comprising exogenous genetic material encoding the antigen. It has been found that, as relates to HIV antigens, administration in accordance with this description results in a significant non-additive synergistic effect which notably increases the immune response seen in inoculated

mammalian hosts. The effects are particularly evident in the cellular immune responses generated following inoculation. The disclosed immunization regime, thus, offers a prophylactic advantage to previously uninfected individuals and can offer a therapeutic effect to reduce viral load levels in those already infected with the virus, hence prolonging the asymptomatic phase of HIV-1 infection.

Accordingly, the instant invention relates to a method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host comprising the steps of (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter (b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; said recombinant poxvirus vector being replication-impaired in the mammalian host. "Replication-impaired" in this context has a broad meaning and generally describes (1) those vectors that have been attenuated or modified such that replication is not possible; (2) those vectors that have been attenuated or modified such that replication is impaired; and (3) those vectors that simply do not replicate, or replicate at a much reduced level, in the particular mammalian species that is treated. Replication of avipoxviruses, for instance, appears to be restricted to avian species. For this reason, avipoxviruses stand as a very safe vector for use in mammals. Replication appears to be blocked at a step prior to viral-DNA synthesis, presumably allowing for the use of only the early promoters; *see, e.g.*, Moss, B., 1993 *Curr. Opin. Genet. Devel.* 3:86-90; and Taylor *et al.*, 1991 *Vaccine* 9:190-3. This level of replication has, however, been noted to afford protective immunization; *see, e.g.*, Wild *et al.*, 1990 *Vaccine* 8:441-442; and 1992 *Virology* 187:321-28; and Cadoz *et al.*, 1992 *Lancet* 339:1429-32. Poxviruses form an essential element of the instant methods as they have been found to exhibit a surprising ability to significantly boost an adenoviral-primed immune response against HIV. Specific embodiments of the instant invention employ modified vaccinia viruses (such as Modified Vaccinia Virus Ankara ("MVA"), subject of U.S. Patent No. 5,185,146; and NYVAC, a highly attenuated strain of vaccinia virus disclosed in, *inter alia*, Tartaglia *et al.*, 1992 *Virology* 188:217-232) in the boosting administrations of the instant invention, although any poxvirus and, particularly vaccinia virus, that can effectuate the delivery and expression of an

antigen of interest and which is of reduced virulence in the intended mammalian host is encompassed herein. Modified vaccinia viruses and their use in various methods have been disclosed in the art, *see, e.g.*, U.S. Patent Nos. 5,185,146; 5,110,587; 4,722,848; 4,769,330; 5,110,587; and 4,603,112. This is true as well for generalized methods for constructing recombinant vaccinia virus; *see, e.g.*, Earl *et al.*, In *Current Protocols in Molecular Biology*, Ausubel *et al.* eds., New York: Greene Publishing Associates & Wiley Interscience; 1991:16.16.1-16.16.7. Further embodiments of the instant application utilize alternative poxvirus vectors in the boosting administration of the disclosed methods. Of specific mention, are avipoxviruses such as ALVAC (the subject of, *inter alia*, U.S. Patent Nos. 5,505,941; 5,174,993; 5,942,235; 5,863,542; and 5,174,993). ALVAC, as indicated earlier, is a plaque-purified clone derived from an attenuated canarypox virus obtained from the wild-type strain after 200 passages in chick embryo fibroblasts. ALVAC recombinants and the use thereof form another aspect of the instant invention. A specific example of such an ALVAC recombinant is vCP 205. vCP 205 (ATCC Acc. No. VR-2547) is, in brief, an ALVAC recombinant (ALVAC-MN120TMG) which expresses HIV1 (IIIB) gag (and protease) proteins, as well as a form of the HIV1(MN) envelope glycoprotein in which gp120 is fused to the transmembrane anchor sequence derived from gp41. Incorporation of the HIV genes in an ALVAC backbone is described in issued U.S. Patent No. 5,863,542 (*see, e.g.*, Example 14). The recombinant canarypox virus ALVAC-HIV (vCP205) was obtained by homologous recombination between the pHIV32 plasmid and the ALVAC genomic DNA. The pHIV32 plasmid encodes the HIV-1 gp120-MN and the anchoring region of gp41 (transmembrane glycoprotein of HIV-1 gp41 LAI), the Gag p55-polyprotein, and the protease-LAI whose expressions are under control of the HG and I3L vaccinia promoters, respectively. The nucleotide sequence of the H6-promoted HIV1 gp120 (+transmembrane) gene and the I3L-promoted HIV1gag(+pro) gene contained in pHIV32 is disclosed in Figures 14A to 14C of U.S. Patent No. 5,863,542 which is hereby incorporated by reference.. Deletion of the ectodomain of gp41 is believed to make it easier to distinguish between infected and vaccinated subjects since most HIV-infected subjects show antibodies directed against the immunodominant region of gp41 precisely deleted in vCP205.

Strategies involved in the construction of recombinant poxvirus are known, *see, e.g.*, Panicali & Paoletti, 1982 *Proc. Natl. Acad. Sci. USA* 79:4927-31; Nakano *et*

al., 1982 *Proc. Natl. Acad. Sci. USA* 79:1593-96; Piccini *et al.*, In *Methods in Enzymology*, Wu & Grossman, eds., Academic Press, San Diego, 153:545-63; U.S. Patent No. 4,603,112; Sutter *et al.*, 1994 *Vaccine* 12:1032-40; and Wyatt *et al.*, 1996 *Vaccine* 15:1451-8. Methods for creating synthetic recombinant poxviruses are also  
5 described in U.S. Patent Nos. 4,769,330; 4,722,848; 4,603,112; 5,110,587; and 5,174,993 ; the disclosures of which are incorporated herein by reference. The construction of recombinant MVA and ALVAC recombinant virus comprising exogenous genetic material coding for HIV gag is described herein in Examples 2 and 10, respectively. As one of ordinary skill in the art will appreciate, insertion of the  
10 exogenous genetic material can be targeted to numerous locations of the poxvirus genome provided the location does not negate the ability of the virus to effect expression of the genetic material. In order to ensure the infectivity of the virus and, hence, expression of the construct, insertion must occur into silent regions of the genome or into nonessential genes. The recombinant MVA constructs disclosed  
15 herein, for instance, have the exogenous genetic material incorporated into the thymidine kinase region and the deletion II region (a region defined, *inter alia*, in Meyer *et al.*, 1991 *J. Gen. Virol.* 72:1031-8); see Example 2.

Recombinant adenoviral vectors form an essential element of the methods of the instant invention as they have been found to very effectively prime the immune  
20 response against a specific antigen of interest. Preferred embodiments of the instant invention employ adenoviral vectors which are replication-defective by reason of having a deletion in/activation of the E1 region which renders the vector devoid (or essentially devoid) of E1 activity. Adenovirus serotype 5 has been found to be a very effective adenovirus vehicle for purposes of effectuating sufficient expression of  
25 exogenous genetic material (particularly HIV antigens) in order to provide for sufficient priming of the mammalian host immune response. Alternative replication-defective adenoviral vehicles capable of effecting expression of the HIV antigen are, however, also suitable for use herein.

The wildtype adenovirus serotype 5 sequence is known and described in the  
30 art; see, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is an immunization scheme employing a vector based on the wildtype adenovirus serotype 5 sequence in the priming administration; a virus of which has been deposited with the American Type Culture Collection ("ATCC") under ATCC Deposit No. VR-5.



One of skill in the art can, however, readily identify alternative adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42) and incorporate same into the disclosed heterologous prime-boost immunization schemes. Accordingly, the instant invention encompasses methods employing all adenoviral vectors partially deleted in.

5 E1 in the administration schemes of the instant invention.

Recombinant adenoviral vectors comprising deletions additional to that contained within the region of E1 are also contemplated for use within the methods of the instant invention. For example, vectors comprising deletions in both E1 and E3 are contemplated for use within the methods of the instant invention. Such a vector  
10 can accommodate a larger amount of foreign DNA inserts (or exogenous genetic material).

Adenoviral vectors of use in the methods of the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in*  
15 *Pharmacology* 40:137-206, which is hereby incorporated by reference.

Adenoviral pre-plasmids (e.g., pMRKAd5gag) can be generated by homologous recombination using adenovirus backbones (e.g., MRKHVE3) and the appropriate shuttle vector. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells, and virus is produced. The infected cells and media are  
20 then harvested after viral replication is complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby  
25 incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>,  
30 from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is preferred that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

Adenoviral and poxvirus vectors of use in the instant invention comprise a gene encoding an HIV-1 antigen or an immunologically relevant modification thereof. HIV antigens of interest include, but are not limited to, the major structural proteins of HIV such as Gag, Pol, and Env, immunologically relevant modifications, and immunogenic portions thereof. The invention, thus, encompasses the various forms of codon-optimized HIV-1 gag (including but by no means limited to p55 versions of codon-optimized full length ("FL") Gag and tPA-Gag fusion proteins), HIV-1 pol, HIV-1 nef, HIV env, and selected modifications of immunological relevance. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous (non-native) or modified native promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription termination sequence; provided that any promoter utilized to drive expression of the nucleic acid included within the gene expression cassette for the recombinant poxvirus vector is either native to, or derived from, the poxvirus of interest or another poxvirus member. Naturally occurring, nonoverlapping, tandem early/late promoters of moderate strength have been described for vaccinia virus (see, e.g., Cochran, *et al.*, 1985 *J. Virol.* 54:30-37; and Rosel *et al.*, 1986 *J. Virol.* 60:436-9) and have been used for gene expression. An example of a modified native promoter is the synthetic early/late promoter of Example 2, previously described in Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-97. Preferably, the gene expression cassette used within the recombinant poxvirus comprises (a) a nucleic acid encoding an HIV antigen (e.g., an HIV protein) or biologically active and/or immunologically relevant portion thereof; and (b) a heterologous promoter (from another poxvirus species) or a promoter which is native to or derived from the poxvirus of interest.

The transcriptional promoter of the recombinant adenoviral vector is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman *et al.*, 1991 *Nucl. Acids Res* 19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV),

constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter. In preferred embodiments, the promoter may comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought. Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:4). A recombinant adenoviral vectors with an expression cassette comprising a CMV promoter (devoid of the intron A region) and a BGH terminator forms a specific aspect of the present invention, although other promoter/terminator combinations can be used. Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA.

Recombinant viral vectors in accordance with the instant disclosure form an aspect of the instant invention. Other aspects of the instant invention are host cells comprising said adenoviral and/or pox virus vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral and/or pox virus vector into a host cell, and (b) harvesting the resultant vectors.

Administration of the viral vectors in accordance with the methods of the instant invention should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen

(e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be incorporated into the recombinant viral vectors of use in the methods of the instant invention, preferred embodiments include the codon optimized p55 gag antigen, pol and nef. The adenoviral and/or pox virus vehicles of the instant invention can utilize heterologous nucleic acid which may or may not be codon-optimized. In specific embodiments of the instant invention, the individual can be primed with an adenoviral vector comprising codon-optimized heterologous nucleic acid, and boosted with a pox virus vector comprising non-codon-optimized nucleic acid. Administration of multiple antigens possesses the possibility for exploiting various different combinations of codon-optimized and non-codon-optimized sequences.

Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the viral vaccines will encode modified versions of pol or nef. Preferred embodiments of the viral vaccines carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. A clade B or clade C based p55 gag antigen will potentially be useful on a global scale. A transgene of choice for insertion into the vectors utilized within the disclosed methods is a codon-optimized version of p55 gag.

In addition to a single HIV antigen of interest being delivered by the adenoviral and poxvirus vectors, two or more antigens can be delivered either via separate vehicles or delivered *via* the same vehicle. For instance, a priming dose in accordance with the instant invention can comprise a recombinant viral vector comprising genes encoding both nef and pol or, alternatively, two or more alternative HIV-1 antigens. The boosting dose could then comprise a recombinant poxvirus vector comprising the genes encoding both nef and pol (or whichever two or more HIV-1 antigens were used in the priming dose). In an alternative scenario, the priming dose can comprise a mixture of separate adenoviral vehicles each comprising a gene encoding for a different HIV-1 antigen. In such a case, the poxvirus boosting dose would also comprise a mixture of poxvirus vectors each comprising a gene encoding for a separate HIV-1 antigen, provided that the boosting dose administers recombinant viral vectors comprising genetic material encoding for the same antigens that were delivered in the priming dose. Alternatively, a poxvirus vector expressing all HIV-1 antigens could be generated to serve as a boosting agent for vaccination. These divalent (*e.g.*, gag and nef, gag and pol, or pol and nef components) or trivalent (*e.g.*, gag, pol and nef components) vaccines can further be administered by a combination of the techniques described above. Therefore, a preferred aspect of the present invention are the various vaccine formulations that can be administered by the methods of the instant invention. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen.

The disclosed immunization regimes employing fusion constructs composed of two or more antigens are also encompassed herein. For example, multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-viral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, or possibly a "2+1" divalent vaccine comprising, for instance, a gag-pol fusion (*e.g.*, a codon optimized p55 gag and inactivated optimized pol) with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames in the same construct may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. In the absence of the use of IRES-based technology, it is

preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may include a three transgene vector such as that wherein a gagpol fusion and nef gene were included in the same vector with different promoters and termination sequences being used for the gagpol fusion and nef gene. Further, potential "2+1" divalent vaccines of the present invention might be wherein a single construct containing gag and nef with separate promoters and termination sequences is administered in combination with a construct comprising a pol gene with promoter and termination sequence. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These compositions are, as above, preferably delivered along with a viral composition comprising an additional HIV antigen in order to diversify the immune response generated upon inoculation. Therefore, a multivalent vaccine delivered in a single, or possibly second, viral vector is certainly contemplated as part of the present invention. It is important to note that, in terms of deciding on an insert for the recombinant adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the viral vehicle. Adenovirus, for instance, has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred in certain embodiments that the sequence be "optimized" for expression in a mammalian (*e.g.*, human cellular environment, particularly in the adenoviral constructs. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon

frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeast and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is a vaccine administration protocol wherein the adenoviral and poxvirus vectors both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol, env, or nef, although as stated above, one or more of the viral vehicles of the instant invention can utilize heterologous nucleic acid which may or may not be codon-optimized. In specific embodiments of the instant invention, the individual can be primed with an adenoviral vector comprising codon-optimized heterologous nucleic acid, and boosted with a pox virus vector comprising non-codon-optimized nucleic acid. Administration of multiple antigens possesses the possibility for exploiting various different combinations of codon-optimized and non-codon-optimized sequences.

A vaccine composition comprising the recombinant viral vectors either in the priming or boosting dose in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation for

the recombinant adenoviral vector has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl<sub>2</sub>; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used to make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl<sub>2</sub>, 0.005% polysorbate 80 at pH 8.0. This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of viral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The administration schemes of the instant invention are based on the priming of the immune response with an adenoviral vehicle comprising a gene encoding an HIV antigen (or antigens) and, following a predetermined length of time, boosting the adenovirus-primed response with a poxvirus vector comprising a gene encoding an HIV antigen(s). Multiple primings, typically, 1-4, are usually employed, although more may be used. The length of time between prime and boost may typically vary from about four months to a year, but other time frames may be used. The booster dose may be repeated at selected time intervals.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV but remain uninfected; CTL has been noted in



several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression.

5

The following non-limiting Examples are presented to better illustrate the invention.

#### EXAMPLE 1

10

##### HIV-1 Gag Gene

A synthetic gene for HIV gag from HIV-1 strain CAM-1 was constructed using codons frequently used in humans; *see* Korber *et al.*, 1998 *Human Retroviruses and AIDS*, Los Alamos Nat'l Lab., Los Alamos, New Mexico; and Lathe, R., 1985 *J. Mol. Biol.* 183:1-12. Figure 2 illustrates the nucleotide sequence of the exemplified optimized codon version of full-length p55 gag. The gag gene of HIV-1 strain CAM-1 was selected as it closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence (Los Alamos HIV database). Advantage of this "codon-optimized" HIV gag gene as a vaccine component has been demonstrated in immunogenicity studies in mice. The "codon-optimized" HIV gag gene was shown to be over 50-fold more potent to induce cellular immunity than the wild type HIV gag gene when delivered as a DNA vaccine.

A KOZAK sequence (GCCACC) was introduced proceeding the initiating ATG of the gag gene for optimal expression. The HIV gag fragment with KOZAK sequence was amplified through PCR from V1Jns-HIV gag vector. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; *see* Montgomery *et al.*, 1993 *DNA Cell Biol.* 12:777-783, for a description of the plasmid backbone.

30

## EXAMPLE 2

Recombinant MVA Construction And Purification

Two recombinant MVA constructs were constructed with the HIV gag gene  
5 fragment with KOZAK sequence cloned into two different locations of the MVA  
genome, the viral thymidine kinase region (MVA-HIV gag TK) and the deletion II  
region (MVA-HIV gag dII), respectively, with the appropriate linker sequence of the  
restriction sites. The thymidine kinase region insertion was achieved through the use  
of shuttle vector pSC59 (*see*, Chakrabarti *et al.*, 1997 *BioTechniques* 23(6):1094-  
10 1097) with the HIV gag fragment inserted at a unique *Xho* I site. The deletion II  
region insertion was accomplished through the use of pLW21 wherein the HIV gag  
fragment was inserted at a unique *Pme*I site. pLW21 is basically a plasmid derived  
from pGEM4 vector (Promega) containing a single synthetic early/late promoter and a  
unique *Pme*I site for cloning. The promoter and cloning site are flanked by MVA  
15 viral sequence on both sides for targeted insertion upon homologous recombination  
events into the deletion II region of the MVA genome. Expression of the transgene  
within both constructs is driven by a synthetic early/late promoter previously  
described for vaccinia virus (Chakrabarti *et al.*, *supra*). Viral transcription termination  
and polyadenylation signal sequences were not included in the inserted fragment, as  
20 sequences native to the flanking regions of the insert were generally considered  
sufficient for the transcription termination and polyadenylation of transgene transcript  
(*see* B Moss, *Current Topics in Microbiology and Immunology*, 158:25, 1992). The  
authenticity of the transgene product expressed through the poxvirus vector was  
guaranteed by the translational termination codon (TAA) at the 3' end of transgene  
25 ORF. The orientation and authenticity of the insertions were confirmed by DNA  
sequencing.

Methods for generating recombinant MVA have been described previously  
(*see, e.g.*, Sutter *et al.*, 1994 *Vaccine* 12:1032-1040; Wyatt *et al.*, 1996 *Vaccine*,  
15:1451-1458). Briefly, sub-confluent primary chick embryo fibroblast cells (CEF) in  
30 25 cm<sup>2</sup> cell culture flask were infected with wild-type MVA at a multiplicity of  
infection ("m.o.i.") of 0.05 for two hours, and were then transfected with  
approximately 20 mcg of shuttle vector DNA precipitated with Lipofectin (GIBCO  
BRL). The cells were cultured for two days, and then the cell pellets were lysed in 1  
ml PBS/BSA by repeated freezing-thawing. The cell lysate was used to infect CEFs

in a 6-well plate at dilutions of 1:3, 1:9 and 1:27 in duplicates. After two days, the medium was removed and the cell monolayers were washed twice with PBS. The cells were then frozen and thawed three times and the plaques containing cells infected with recombinant MVA were identified by immunostaining, with sequential incubations with a monoclonal antibody against HIV gag (Advanced Biotechnology Inc) and goat-anti-mouse IgG antibody conjugated with peroxidase (Pierce) with *o*-dianisidine as substrate. The blue plaques formed by the infected cells were picked under the inverted microscope, and the cells were diluted in 1 ml PBS. The cells were lysed by freezing-thawing, and the recombinant MVA was further purified in CEF, using dilutions of 1:5, 1:20 and 1:80, for another 5 rounds. The recombinant MVA was then expanded in CEF in a tissue culture flask of 25 cm<sup>2</sup>, and the expression of HIV gag was confirmed by Western blot analysis in CV-1 cells infected with MVA at different dilutions. The final viral stock was prepared in 40 to 80 flasks of 150 cm<sup>2</sup> of CEF, and the viral titers were determined by plaque assay using an immunostaining method.

Recombinant MVA constructs with insertion into the deletion II region were used in the immunizations discussed below.

### EXAMPLE 3

#### Generation of Adenoviral Vector Constructs

##### A. Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *BglII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglII*. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA

expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pV1JnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *Bgl*III digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *Bgl*III site. Colonies were screened using *Sma*I restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHPA, was fully sequenced to confirm sequence integrity.

#### 10 B. Construction of the Modified Shuttle Vector -"MRKpdeIE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from base pairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- 15 (1) The left ITR region was extended to include the *Pac*I site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
- 20 (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbone pAdHVE3 by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

#### 30 C. Construction of Modified Adenovector Backbone

An original adenovector pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region) was reconstructed so that it would contain the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *Pac*I and *Bst*Z1101 and

isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from the transformation were selected and grown in Terrific™ broth for 6-8 hours until  
5 turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from the transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovector was designated MRKpAdHVE3 (E3+ plasmid). Virus from the new adenovector (MRKHVE3) as  
10 well as the old version were generated in the PER.C6® cell lines. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I, *Bam*HI, *Xho* I, *Eco*RV, *Hind*III, *Sal* I, and *Bgl* II sites. This MCS was replaced with a new MCS containing *Not* I, *Cla* I, *Eco*RV and *Asc* I sites. This new MCS has been transferred to the MRKpAdHVE3 pre-plasmid along with the modification made to the  
15 packaging region and pIX gene.

D. Construction of the new shuttle vector containing modified gag transgene – “MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested  
20 with *Msc*I overnight and then digested with *Sfi*I for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 minutes at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 minutes at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by  
25 digestion with *Eco*RV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel orientation.

30

E. Construction of the MRK FG Adenovector

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*I. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified

by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*I overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml  
5 Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH<sub>2</sub>O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from the transformation was selected and grown overnight in 3 ml LB +100  
10 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size.

15 F. Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

MRK Ad5 HIV-1 gag contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

20 The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac*I to release the vector backbone and 3.3 µg was transfected by the calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was  
25 used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two  
30 bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [<sup>33</sup>P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried

down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

5

All viral constructs (adenovirus and poxvirus) were confirmed for Gag expression by Western blot analysis.

#### EXAMPLE 4

10

##### Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered intramuscularly ("i.m.") in 0.5-  
15 mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in  
20 the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

#### EXAMPLE 5

##### ELISPOT Assay

25

The IFN- $\gamma$  ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-  
30 amino acid ("aa") peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50  $\mu$ L of  $2-4 \times 10^5$  peripheral blood mononuclear cells (PBMCs) were added. The cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50  $\mu$ L of media or the gag peptide pool at 8  $\mu$ g/mL concentration per  
35 peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO<sub>2</sub> for 20-24 hrs. Spots were developed accordingly and the plates were processed using

custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD). The counts were normalized to  $10^6$  cell input.

#### EXAMPLE 6

##### Anti-p24 ELISA

5

A modified competitive anti-p24 assay was developed using reagents from the Coulter p24 Antigen Assay kit (Beckman Coulter, Fullerton, CA). Briefly, to a 250- $\mu$ L serum sample, 20  $\mu$ L of Lyse Buffer and 15  $\mu$ L of p24 antigen (9.375 pg) from the  
10 Coulter kit were added. After mixing, 200  $\mu$ L of each sample were added to wells coated with a mouse anti-p24 mAb from the Coulter kit and incubated for 1.5 hr at 37°C. The wells were then washed and 200  $\mu$ L of Biotin Reagent (polyclonal anti-p24-biotin) from the Coulter kit was added to each well. After a 1 hr, 37°C  
15 incubation, detection was achieved using streptavidin-conjugated horseradish peroxidase and TMB substrate as described in the Coulter Kit. OD<sub>450nm</sub> values were recorded. A 7-point standard curve was generated using a serial 2-fold dilution of serum from an HIV-seropositive individual. The lower cut-off for the assay is arbitrarily set at 10 milli Merck units/mL (mMU/mL) defined by a dilution of the seropositive human serum. This cutoff falls at approximately 65% of the maximum  
20 bound control signal which corresponds to that obtained with the diluent control only and with no positive analyte.

#### EXAMPLE 7

##### Intracellular Cytokine Staining

25

To 1 ml of  $2 \times 10^6$  PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal  
30 antibodies were added to a final concentration of 1  $\mu$ g/mL. For gag-specific stimulation, 10  $\mu$ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20  $\mu$ L of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hours at 37 °C, 5% CO<sub>2</sub>, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were  
35 pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20



5  $\mu$ L per tube anti-hCD3-APC, clone FN-18 (Biosource); 20  $\mu$ L anti-hCD8-PerCP,  
 clone SK1 (Becton Dickinson); and 20  $\mu$ L anti-hCD4-PE, clone SK3 (Becton  
 Dickinson). Sample handling from this stage was conducted in the dark. The cells  
 were washed and incubated in 750  $\mu$ L 1xFACS Perm buffer (Becton Dickinson) for  
 10 minutes at room temperature. The cells were pelleted and re-suspended in  
 PBS/2%FBS and 0.1  $\mu$ g of FITC-anti-hIFN- $\gamma$ , clone MD-1 (Biosource) was added.  
 After 30 minutes of incubation, the cells were washed and re-suspended in PBS.  
 Samples were analyzed using all four color channels of the Becton Dickinson FACS  
 Calibur instrument. To analyze the data, the low side- and forward-scatter  
 10 lymphocyte population was initially gated and a common fluorescence cut-off for  
 cytokine-positive events was used for both CD4<sup>+</sup> and CD8<sup>+</sup> populations, and for both  
 mock and gag-peptide reaction tubes of a sample.

## EXAMPLE 8

### Results

#### A. Immunization Regimen

Cohorts of 3-6 rhesus macaques were immunized following homologous and  
 heterologous prime-boost regimens involving MRKAd5 and MVA vectors expressing  
 the same codon-optimized HIV-1 gag. The immunization schedule is described in  
 20 Table 1.

**Table 1**

Group	Prime	Boost (month 6)
1	10e9 vp MRKAd5-HIVgag at week 0, 4	10e9 vp MRKAd5-HIVgag
2	10e9 pfu MVA-HIVgag at week 0, 4	10e9 pfu MVA-HIVgag
3	10e9 vp MRKAd5-HIVgag at week 0, 4	10e9 pfu MVA-HIVgag

#### B. T Cell Immune Responses

25 Vaccine-induced T cell responses against HIV-1 gag were quantified using  
 IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the  
 entire protein sequence. The results are shown in Figures 5 and 6. They are  
 expressed as the number of spot-forming cells (SFC) per million peripheral blood  
 30 mononuclear cells (PBMCs) that responded to the peptide pool minus the mock  
 control.

Figure 5 shows the T cell responses induced by (a) two priming immunizations with 10e9 vp MRKAd5 HIV-1 gag followed by a 10e9 vp MRKAd5 HIV-1 gag booster ("10e9 vp MRKAd5-10e9 vp MRKAd5"); (b) two priming doses of 10e9 pfu MVA HIV-1 gag and a single booster with 10e9 pfu MVA HIV-1 gag ("10e9 pfu MVA-10e9 pfu MVA"); or (c) two priming doses of 10e9 vp of MRKAd5 HIV-1 gag followed by a single booster shot with 10e9 pfu MVA HIV-1 gag ("10e9 vp MRKAd5-10e9 pfu MVA"). The rest period between last priming and booster doses varied from 20-23 weeks (20 for the MVA-MVA subjects; 22 for subjects 99D262, 99C117, and 99D227 of the MRKAd5-MRKAd5 group; and 23 for the remaining subjects). Administration of the same dose of MRKAd5 HIV-1 gag at approximately month 6 resulted in slight increases compared to the levels just prior to the boost; the post-boost levels were largely comparable to if not weaker than the peak levels before the boost. This is possibly due to the presence of neutralizing immunity generated against the vector by the first two immunizations. The responses after the boost did not surpass 500 gag-specific T cells per 10e6 PBMC, with a mean of 275 SFC/10e6 PBMC for all 6 monkeys. Monkeys given three of 10e9 pfu MVA HIV-1 gag (at 0, 1, 6 months) exhibited very weak HIV-specific T cells responses not exceeding 100 SFC/10e6 PBMC. In contrast, when both modalities are combined in which animals were given two priming doses of 10e9 vp MRKAd5 HIV-1 gag and a single booster shot of 10e9 pfu MVA HIV-1 gag, the levels of gag-specific T cells increased to peak responses above 1200 SFC/10e6 PBMC for all 3 monkeys. The property of MVA HIV-1 gag to boost effectively MRKAd5-gag-primed immune responses is very striking considering that MVA HIV-1 gag is a rather poor immunogen; it also offers a great advantage compared to boosting with the same MRKAd5 HIV-1 gag. The ability of poxvirus vector to boost primed responses was also evident using a lower priming dose of 10<sup>7</sup> vp of MRKAd5 HIV-1 gag (Figure 6).

PBMCs from the vaccinees of the heterologous MRKAd5 prime-MVA boost regimen were analyzed for intracellular IFN- $\gamma$  staining after the priming immunizations (week 13) and after the booster immunizations (wk 31). The assay provided information on the relative amounts of CD4<sup>+</sup> and CD8<sup>+</sup> gag-specific T cells in the peripheral blood (Table 2). The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

Table 2

Prime	Boost	ID	Post Prime		Post Boost	
			%CD4+	%CD8+	%CD4+	%CD8+
MRKAd5-HIVgag	MVA-HIVgag	99D241	0.00*	0.13	0.08**	0.37**
10 <sup>9</sup> vp	10 <sup>9</sup> pfu	99D244	0.02	0.09	0.25	0.92
wk 0, 4	wk 27	99D252	0.04	0.08	0.43	0.13

Numbers reflect the percentages of circulating CD3+ lymphocytes that are either gag-specific CD4+ or gag-specific CD8+ cells. Mocks values have been subtracted.

\*No detectable antigen-specific CD4+ T cells above background

\*\*Collected at wk 35 instead of wk 31

### C. Humoral Immune Responses

The p24-specific antibody titers were determined for each animal at several time points. The geometric mean titers for each cohort were calculated and shown in Figure 10. Two doses of MRKAd5 HIV-1 gag were able to induce moderate levels of anti-p24 antibodies (about 1000 mMU/mL) whereas two doses of MVA did not appear to induce any detectable level of anti-p24 antibodies. Administration of MVA HIV-1 gag boosted the humoral immune responses primed by MRKAd5 HIV-1 gag by about 6-fold (to about 7000 mMU/mL). This booster effect is similar to that elicited by a 10<sup>9</sup> vp dose of MRKAd5 HIV-1 gag. However, the booster effect seen in these animals with 10<sup>9</sup> vp MRKAd5 HIV-1 gag is expected to be lower if the subjects have higher levels of Ad5-directed neutralizing activity due to anamnestic responses to the first two MRKAd5 doses. The booster effect of MVA HIV-1 gag, on the other hand, would not be affected by any pre-existing neutralizing titers directed at Ad5.

### EXAMPLE 9

#### Immunization Regime Using Replication-Proficient Vaccinia Virus

BALB/c mice were vaccinated intramuscularly with one of the following immunization regimes: (1) one priming dose of 5x10<sup>8</sup> vp Ad5-gag (the adenoviral vector disclosed in PCT International Application No. PCT/US00/18332 which is hereby incorporated by reference); (2) one priming dose of 5x10<sup>8</sup> vp Ad5-gag followed by one boosting dose of 5x10<sup>6</sup> pfu vaccinia-gag; or (3) one priming dose of 5x10<sup>6</sup> pfu vaccinia-gag. The response in totally naïve animals was also assayed. Figure 7 shows the mock-corrected frequencies of T cells specific for a defined gag CD8+ epitope in BALB/c mice (AMQMLKETI). The results indicate that the Ad5-

primed immune responses (about 300 per million) were boosted significantly by administration of vaccinia-gag (to about 1400 per million).

While this virus is replication-competent and hence not suitable for use in the methods of the instant invention (absent modification), Applicants believe that the example serves to demonstrate with a different poxvirus strain how poxvirus very effectively boosts an adenovirus-primed response.

The mice in this example, one will note, were only primed once. Those of skill in the art will appreciate that due consideration must be given to the general observation that these smaller animal systems require less number of immunizations and/or smaller doses to prime the immune compared to larger non-human primates.

#### EXAMPLE 10

##### Recombinant ALVAC Construction And Purification

Recombinant ALVAC constructs expressing the codon-optimized human HIV-1 gag open reading frame (SEQ ID NO: 1) were generated in accordance with basic procedure well understood and appreciated in the art; *see, e.g.*, U.S. Patent Nos. 5,863,542 and 5,766,598. The procedure generally entails the placement of a gene sequence of interest (herein, SEQ ID NO: 1) ligated or operatively linked to a promoter of interest (e.g., H6 vaccinia virus early promoter) into a plasmid construct containing DNA homologous to a section of DNA within the poxvirus where insertion is desired. As previously mentioned, this site should not contain an essential locus. Following this first step(s), the resulting plasmid construct is amplified by growth within *E. coli* bacteria and isolated. The isolated plasmid containing the insert of interest is then transfected into a cell culture, *e.g.*, chick embryo fibroblasts, along with the pox virus of interest (herein, ALVAC). The recombinant viruses are then selected and purified by serial rounds of plaque purification.

#### EXAMPLE 11

##### Generation of Adenoviral Serotype 6 Vector Constructs

##### A. Construction of Ad6 Pre-Adenovirus Plasmid

An Ad6 based pre-adenovirus plasmid which could be used to generate first generation Ad6 vectors was constructed taking advantage of the extensive sequence

homology (approx. 98%) between Ad5 and Ad6. Homologous recombination was used to clone wtAd6 sequences into a bacterial plasmid.

The general strategy used to recover pAd6E1-E3+ as a bacterial plasmid is illustrated in Figure 11. Cotransformation of BJ 5183 bacteria with purified wt Ad6 viral DNA (ATCC Accession No. VR-6) and a second DNA fragment termed the Ad5 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 33798 to 35935) and left (bp 1 to 341 and bp 3525 to 5767) end of the Ad5 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad5 342 to 3524. The Ad5 sequences in the ITR cassette provide regions of homology with the purified Ad6 viral DNA in which recombination can occur.

Potential clones were screened by restriction analysis and one clone was selected as pAd6E1-E3+. This clone was then sequenced in its entirety. pAd6E1-E3+ contains Ad5 sequences from bp 1 to 341 and from bp 3525 to 5548, Ad6 bp 5542 to 33784, and Ad5 bp 33967 to 35935 (bp numbers refer to the wt sequence for both Ad5 and Ad6). pAd6E1-E3+ contains the coding sequences for all Ad6 virion structural proteins which constitute its serotype specificity.

## 20 B. Construction of an Ad6 Pre-Adenovirus Plasmid containing the HIV-1 gag gene

### (1) Construction of Adenoviral Shuttle Vector:

The shuttle plasmid MRKpdeIE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was constructed by inserting a synthetic full-length codon-optimized HIV-1 gag gene into MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.).

25 MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) contains Ad5 sequences from bp 1 to 5792 with a deletion of E1 sequences from bp 451 to 3510. The HCMV promoter and BGH pA were inserted into the E1 deletion in an E1 parallel orientation with a unique BglII site separating them. The synthetic full-length codon-optimized HIV-1 gag gene was obtained from plasmid pV1Jns-HIV-FLgag-opt by BglII

30 digestion, gel purified and ligated into the BglII restriction endonuclease site in MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.), generating plasmid MRKpdeIE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA. The genetic structure of MRKpdeIE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was verified by PCR, restriction enzyme and DNA sequence analyses.

(2) Construction of pre-adenovirus plasmid:

Shuttle plasmid MRKpdeE1(Pac/pIX/pack450)+CMVminFL-gag-BGHpA was digested with restriction enzymes *Pac*I and *Bst*1107I and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I-digested) adenoviral backbone plasmid, pAd6E1-E3+. The genetic structure of the resulting pMRKAd6gag was verified by restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for large-scale production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the gag transgene in transient transfection cell culture.

pMRKAd6gag contains Ad5 bp 1 to 450 and from bp 3511 to 5548, Ad6 bp 5542 to 33784, and Ad5 bp 33967 to 35935 (bp numbers refer to the wt sequence for both Ad5 and Ad6). In the plasmid the viral ITRs are joined by plasmid sequences that contain the bacterial origin of replication and an ampicillin resistance gene.

C. Generation of research-grade recombinant MRKAd6gag

To prepare virus for pre-clinical immunogenicity studies, the pre-adenovirus plasmid pMRKAd6gag was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 10 µg of pMRKAd6gag was digested with restriction enzyme *Pac*I (New England Biolabs) and transfected into a 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested after complete viral cytopathic effect (CPE) was observed. The virus stock was amplified by multiple passages in PER.C6<sup>®</sup> cells. At the final passage virus was purified from the cell pellet by CsCl ultracentrifugation. The identity and purity of the purified virus was confirmed by restriction endonuclease analysis of purified viral DNA and by gag ELISA of culture supernatants from virus infected mammalian cells grown in vitro. For restriction analysis, digested viral DNA was end-labeled with P<sup>33</sup>-dATP, size-fractionated by agarose gel electrophoresis, and visualized by autoradiography.

All viral constructs were confirmed for Gag expression by Western blot analysis.

## EXAMPLE 12

Immunization

5 Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points (typically, four week intervals) during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

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## EXAMPLE 13

ELISPOT Assay

20 The IFN- $\gamma$  ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749; Casimiro *et al.*, 2002 *J. Virol.* 76:185-94), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-amino acid ("aa") peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50  $\mu$ L of  $2-4 \times 10^5$  peripheral blood mononuclear cells (PBMCs) were added. The cells were counted using a Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50  $\mu$ L of media or the gag peptide pool at 8  $\mu$ g/mL concentration per peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO<sub>2</sub> for 20-24 hrs. Spots were developed accordingly and counted under microscope. The counts were normalized to  $10^6$  cell input.

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## EXAMPLE 14

Intracellular Cytokine Staining

35 To 1 ml of  $2 \times 10^6$  PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293,

Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 µg/mL. For gag-specific stimulation, 10 µL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hour, after which 20 µL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hours at 37 °C, 5% CO<sub>2</sub>, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 minutes at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 minutes, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 µL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 µL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 µL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 µL 1xFACS Perm buffer (Becton Dickinson) for 10 minutes at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 µg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 minutes of incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACS Calibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated and a common fluorescence cut-off for cytokine-positive events was used for both CD4<sup>+</sup> and CD8<sup>+</sup> populations, and for both mock and gag-peptide reaction tubes of a sample.

## EXAMPLE 15

### Results

#### 25 A. Immunization Regimen

A cohort of four (4) macaques were given three (3) doses of either MRKAd5-HIVgag or MRKAd6-HIVgag at weeks 0, 4, 26. At week fifty-six (56), a booster shot of 10<sup>8</sup> pfu of ALVAC-HIVgag was delivered intramuscularly. For comparison, a separate cohort of three (3) monkeys were given three (3) doses of the same ALVAC-HIVgag (10<sup>9</sup> pfu) at weeks 0, 4, 27. All viral vectors expressed the same codon-optimized HIV-1 gag. The immunization schedule is described in Table 3.



**Table 3**

Grp	Monkey ID	Vaccine 1	Vaccine 2
1	99C117	10 <sup>9</sup> vp MRKAd5-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56
	99D021	10 <sup>7</sup> vp MRKAd5-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56
	99D126	10 <sup>9</sup> vp MRKAd6-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56
	99D147	10 <sup>7</sup> vp MRKAd6-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56
2	127F, 57T, 84TX	10 <sup>9</sup> pfu ALVAC-HIVgag at wk 0, 4, 27	none

**B. T Cell Immune Responses**

Vaccine-induced T cell responses against HIV-1 gag were quantified using an IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 12. They are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool minus the mock control.

Figure 12 shows that 10<sup>7</sup>-10<sup>9</sup> vp dose of MRKAd5-HIVgag or MRKAd6-HIVgag induced levels of gag-specific T cell responses not exceeding 600 SFC/10<sup>6</sup> PBMC. Three out of the four animals had levels below 300 SFC/10<sup>6</sup> PBMC after two doses of the adenoviral-based vaccine. At the time of the ALVAC booster immunization which is about half a year since the last adenovirus dose, antigen-specific responses remained detectable ranging from 10-114 SFC/10<sup>6</sup> PBMC in these animals. However, administration of the ALVAC resulted in about 10-80-fold enhancement in T cell responses when compared to the levels at the time of the booster. These results are very surprising given that ALVAC is intrinsically a rather weak vaccine vector for inducing primary T cell immune response in macaques. Three monkeys that were given multiple immunizations of ALVAC-HIVgag at an even higher dose level (10<sup>9</sup> pfu) exhibited very weak responses to the antigen (less than 100 SFC/10<sup>6</sup> PBMC) (Figure 12).

It is not believed that a fourth immunization with the same adenovirus at an equivalent dose level such as that provided the first three (3) times would be capable of eliciting these large responses because of the potentially significant pre-existing anti-adenovirus immunity generated by the first three (3) doses. Also note that the third adenovirus dose in these monkeys yielded levels that do not even compare to the levels seen following the ALVAC booster. These results clearly show that while ALVAC-based vectors are weak inducers of primary immune response they serve as excellent boosters of existing immune response to an HIV antigen. It also illustrates that a synergy exists between MRKAd-based vectors and ALVAC.

PBMCs from the vaccinees of the heterologous MRKAd5/MRKAd6-ALVAC boost regimens were analyzed for intracellular IFN- $\gamma$  staining after the boosting immunization (week 60). The assay results provide information on the relative amounts of CD4<sup>+</sup> and CD8<sup>+</sup> gag-specific T cells in the peripheral blood (Table 4).

- 5 The results indicate that the heterologous prime-boost immunization approach was able to elicit both HIV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in rhesus macaques.

**Table 4**

Monkey ID	Vaccine 1	Vaccine 2	Gag-Specific (Wk 60)	
			%CD4	%CD8
99C117	10 <sup>9</sup> vp MRKAd5-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56	0.12	0.26
99D021	10 <sup>7</sup> vp MRKAd5-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56	0.08	0.70
99D126	10 <sup>9</sup> vp MRKAd6-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56	0.06	0.35
99D147	10 <sup>7</sup> vp MRKAd6-HIVgag at wk 0, 4, 26	10 <sup>8</sup> pfu ALVAC-HIVgag at wk 56	0.07	0.23

- 10 Numbers reflect the percentages of circulating CD3<sup>+</sup> lymphocytes that are either gag-specific CD4<sup>+</sup> or gag-specific CD8<sup>+</sup> cells. Mocks values (less than 0.02%) have been subtracted.

## EXAMPLE 16

### Immunization and Results

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#### A. Immunization

- Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.
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#### B. ELISPOT Assay

- The IFN- $\gamma$  ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50  $\mu$ L of 2-4 x 10<sup>5</sup> peripheral
- 30

blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 fL. Either 50  $\mu$ L of media or the gag peptide pool at 8  $\mu$ g/mL concentration per peptide were added to the PBMC. The samples were incubated at 37°C, 5% CO<sub>2</sub> for 20-24 hrs. Spots  
5 were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10<sup>6</sup> cell input.

#### C. Intracellular Cytokine Staining

To 1 ml of 2 x 10<sup>6</sup> PBMC/mL in complete RPMI media (in 17x100mm  
10 round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1  $\mu$ g/mL. For gag-specific stimulation, 10  $\mu$ L of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20  $\mu$ L of 5 mg/mL of brefeldin A  
15 (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO<sub>2</sub>, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20  $\mu$ L per tube anti-hCD3-APC, clone FN-18 (Biosource); 20  $\mu$ L anti-hCD8-PerCP,  
20 clone SK1 (Becton Dickinson); and 20  $\mu$ L anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750  $\mu$ L 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1  $\mu$ g of FITC-anti-hIFN- $\gamma$ , clone MD-1 (Biosource) was added.  
25 After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both CD4<sup>+</sup> and CD8<sup>+</sup> populations, and for both mock and gag-  
30 peptide reaction tubes of a sample.

#### D. Results

Cohorts of 4 monkeys were given at wk 0 one of the following booster vaccines: (A) ALVAC vcp205, 10<sup>8</sup> pfu; (B) ALVAC vcp205, 10<sup>7</sup> pfu; (C) ALVAC HIV-1 gag, 10<sup>8</sup> pfu; (D) ALVAC HIV-1 gag, 10<sup>7</sup> pfu, or (E) MRKAd5

HIV-1 gag,  $10^9$  vp. ALVAC vcp205 encodes the gene for HIV-1 III<sub>B</sub> gag. ALVAC HIV-1 gag encodes the codon-optimized HIV-1 CAM-1 gag. The animals prior to this immunization had received 3 previous doses of at least  $10^9$  vp Ad5 HIV-1 gag. The last immunization with Ad5 HIV-1 gag was given more than a year prior. The neutralization titers to Ad5 vector were measured in all animals just prior to wk 0 time point. Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN- $\gamma$  ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Table 6; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool minus the mock control.

Table 5

Grp	Booster, Wk 0	Monk ID#	Diff. Days <sup>a</sup>	Ad5 neut <sup>b</sup>	IFN- $\gamma$ ELISPOT, SFC/ $10^6$ PBMC					
					Peak, Prime <sup>c</sup>		T=0 Wk		T=2 Wk	
					Mock	Gag	Mock	Gag	Mock	Gag
1	ALVAC vcp205 $10^8$ pfu	99C069	617	1065	0	116	0	40	1	584
		98X012	848	457	1	121	3	8	3	843
		CB4B	695	285	10	330	3	59	15	865
		98X011	695	192	1	361	10	43	3	1205
		<i>Mean<sup>d</sup></i>	<b>714</b>	<b>404</b>		<b>200</b>		<b>25</b>		<b>841</b>
2	ALVAC HIV-1 gag $10^8$ pfu	99D193	617	291	4	146	0	34	10	1648
		CD1V	617	222	16	251	0	18	13	826
		CB56	617	171	0	265	1	18	5	734
		97N144	848	947	5	373	3	159	0	1838
		<i>Mean<sup>d</sup></i>	<b>675</b>	<b>320</b>		<b>239</b>		<b>35</b>		<b>1156</b>
3	MRKAd5-gag $10^9$ vp	101H	695	490	0	115	3	58	1	696
		99C213	617	98	11	226	3	14	0	420
		99D137	617	754	8	268	4	49	0	1220
		105F	695	507	5	380	15	76	13	163
		<i>Mean<sup>d</sup></i>	<b>656</b>	<b>368</b>		<b>222</b>		<b>36</b>		<b>480</b>

<sup>a</sup>Difference in days between the day of ALVAC boost and the third Ad5 vaccination

<sup>b</sup>Neutralization titers 1 month prior to boost; reported are geometric means of up to 3 measurements

<sup>c</sup>Peak anti-gag T cell responses (SFC/ $10^6$  PBMC) during Ad5 priming vaccinations

<sup>d</sup>Arithmetic means for difference in days; geometric means for Ad5 neut titers; mock-corrected gag T cell responses.

Table 5 shows the T cell responses induced using a homologous boost with MRKAd5-gag or with ALVAC vector. On the basis of the ELISPOT results, it appears that the boosting with ALVAC, specifically ALVAC HIV-1 gag, provides greater booster responses than the MRKAd5-gag.

PBMCs from the vaccinees were analyzed for intracellular IFN- $\gamma$  staining 2 wks after the booster immunization. This assay provided information on the amounts of CD4<sup>+</sup> and CD8<sup>+</sup> gag-specific T cells in the peripheral blood (Table 6).

The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4+ and CD8+ T cells. It also indicates that the ALVAC booster induces as much gag-specific CD8+ T cells as MRKAd5gag. However, the ALVAC booster induces higher levels of helper responses than MRKAd5-gag. On the basis of total antigen-specific CD3+ T cells induced as measured by this assay, the ALVAC HIV-1 gag booster shows a statistically significant 6-fold improvement ( $P=0.004$ ) than the MRKAd5-gag booster.

Table 6

Group	Vaccine	Monk #	CD3+CD4+IFN $\gamma$ + per 10 <sup>6</sup> Lymp <sup>a</sup>		CD3+CD8+IFN $\gamma$ + per 10 <sup>6</sup> Lymp <sup>b</sup>		%CD3+CD8+ <sup>c</sup>	Total CD3+ 10 <sup>6</sup> Lymp <sup>d</sup>
			Mock	Gag	Mock	Gag		
1	ALVAC gag vcp205 10 <sup>8</sup> pfu	99C069	129	945	64	482	33.8	1234
		98X012	17	1160	50	368	21.7	1460
		CB4B	82	1507	100	1203	43.6	2528
		98X011	37	1833	74	656	24.5	2377
		Mean <sup>e</sup>		1243		540		1783
2	ALVAC HIV-1 gag 10 <sup>8</sup> pfu	99D193	87	6744	104	9479	58.5	16032
		CD1V	0	1877	72	702	25.1	2507
		CB56	16	1123	63	2148	65.3	3192
		97N144	60	2231	77	5323	70.7	7417
		Mean <sup>e</sup>		2341		2835		5176
3	MRKAd5 HIV-1 gag 10 <sup>9</sup> vp	101H	62	268	71	643	73.5	778
		99C213	19	245	46	538	68.4	718
		99D137	25	158	58	3592	96.4	3666
		105F	34	218	17	218	52.2	384
		Mean <sup>e</sup>		184		668		852

<sup>a</sup>Number of IFN- $\gamma$  producing CD3+CD4+ cells per million lymphocytes

<sup>b</sup>Number of IFN- $\gamma$  producing CD3+CD8+ cells per million lymphocytes

<sup>c</sup>Percentage of Gag-Specific T cells that are CD3+CD8+

<sup>d</sup>Sum of IFN- $\gamma$  producing CD3+CD4+ plus CD3+CD8+ cells per million lymphocytes

<sup>e</sup>Geometric means of mock-corrected values

### EXAMPLE 17

#### Immunization Regimen

Cohorts of 3-6 rhesus macaques will be immunized in accordance with the following homologous and heterologous prime-boost immunization schedule (Table 7), involving Ad5-gag, -pol, and -nef vectors expressing codon-optimized HIV-1 gag, pol and nef, respectively, and ALVAC-gag, pol, nef expressing all three genes in one virus under separate promoter controls. The total dose of each vaccine will be suspended in approximately 1 mL of buffer. The macaques will be anesthetized (ketamine/xylazine) and the vaccines will be delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson,

- Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) will be prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment will be in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

**Table 7.**

Group	Prime	Boost
1	10 <sup>9</sup> vp/vector Ad5-gag, -pol, -nef at week 0,4	10 <sup>8</sup> pfu ALVAC-gag,pol,nef
2	10 <sup>7</sup> vp/vector Ad5-gag, -pol, -nef at week 0,4	10 <sup>8</sup> pfu ALVAC-gag,pol,nef
3	10 <sup>8</sup> pfu ALVAC-gag,pol,nef at week 0,4	10 <sup>7</sup> vp/vector Ad5-gag, -pol, -nef
4	10 <sup>9</sup> vp/vector Ad5-gag, -pol, -nef at week 0,4	10 <sup>9</sup> vp/vector Ad5-gag, -pol, -nef
5	10 <sup>7</sup> vp/vector Ad5-gag, -pol, -nef at week 0,4	10 <sup>7</sup> vp/vector Ad5-gag, -pol, -nef
6	10 <sup>8</sup> pfu ALVAC-gag,pol,nef at week 0,4	10 <sup>8</sup> pfu ALVAC-gag,pol,nef

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**EXAMPLE 18****SIV Challenge Experiment**

- Cohorts of 3-6 monkeys will be immunized in accordance with the following heterologous prime-boost immunization schedule (Table 8), involving Ad5-SIV-gag, -pol, and -nef vectors expressing codon-optimized SIV gag, pol and nef, respectively, and ALVAC-SIV gag, pol, nef expressing all three genes in one virus under separate promoter controls. The animals will be pre-screened and distributed for the presence of mamuA01 allele. The total dose of each vaccine will be suspended in approximately 1 mL of buffer. The macaques will be anesthetized (ketamine/xylazine) and the vaccines will be delivered intramuscularly ("i.m.") in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) will be prepared from blood samples collected at several time points during the immunization regimen to monitor for SIV-specific T cell responses. After the ALVAC booster, animals will

- be given systemic inoculation of SIVmac239 strain. Animals will be monitored for both virological (i.e., viral loads) and immune parameters (e.g., virus-specific T cell responses, CD4 counts, and lymphoid structures). All animal care and treatment will be in accordance with standards approved by the Institutional Animal Care and Use
- 5 Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

**Table 8.**

<b>Monkey</b>	<b>Prime</b>	<b>Boost</b>	<b>Challen</b>
MamuA01+	10 <sup>11</sup> vp/vector Ad5-SIVgag, -SIVpol, -SIVnef at week 0,4	10 <sup>8</sup> pfu ALVAC-SIVgag,pol,nef at week 24	SIVmac at week
MamuA01+	None	None	SIVmac at week
MamuA01-	10 <sup>11</sup> vp/vector Ad5-SIVgag, -SIVpol, -SIVnef at week 0,4	10 <sup>8</sup> pfu ALVAC-SIVgag,pol,nef at week 24	SIVmac at week
MamuA01-	None	None	SIVmac at week

10

## WHAT IS CLAIMED IS:

1. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:
  - (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter
  - (b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding the HIV-1 antigen or immunologically relevant modification thereof; provided said poxvirus vector is replication-impaired in the mammalian host.
2. A method in accordance with claim 1 wherein the adenoviral vector is of serotype 5.
3. A method in accordance with claim 2 wherein the recombinant adenoviral vector is deleted of base pairs corresponding to base pairs 451-3510 of a wildtype adenovirus serotype 5 genome.
4. A method in accordance with claim 1 wherein the adenoviral vector is of serotype 6.
5. A method in accordance with claim 1 wherein at least one of the genes encoding the HIV-1 antigen or immunologically relevant modification thereof comprises codons optimized for expression in a human.
6. A method in accordance with claim 1 wherein the recombinant adenoviral vector comprises a gene expression cassette comprising:
  - (a) a nucleic acid encoding an HIV-1 antigen;
  - (b) a heterologous promoter operatively linked to the nucleic acid encoding the antigen; and
  - (c) a transcription termination sequence.



7. A method in accordance with claim 1 wherein the recombinant poxvirus vector comprises a gene expression cassette comprising:
- (a) a nucleic acid encoding an HIV-1 antigen; and
  - (b) a promoter operatively linked to the nucleic acid encoding the antigen; provided that said promoter is derived from or native to a poxvirus.
8. A method in accordance with claim 6 wherein the gene expression cassette in the recombinant adenoviral vector is inserted into the E1 region.
9. A method in accordance with claim 8 wherein the gene expression cassette in the recombinant adenoviral vector is in an E1 parallel orientation.
10. A method in accordance with claim 6 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
11. A method in accordance with claim 10 wherein the promoter is an immediate early human cytomegalovirus promoter.
12. A method in accordance with claim 7 wherein the promoter is a synthetic early/late promoter of vaccinia virus.
13. A method in accordance with claim 6 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
14. A method in accordance with claim 6 wherein the HIV-1 antigen is HIV-1 gag.
15. A method in accordance with claim 7 wherein the HIV-1 antigen is HIV-1 gag.
16. A method in accordance with claim 6 wherein the gene expression cassette comprises an open reading frame encoding an HIV-1 gag protein or immunologically relevant modification thereof.

17. A method in accordance with claim 7 wherein the gene expression cassette comprises an open reading frame encoding an HIV-1 gag protein or immunologically relevant modification thereof.

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18. A method in accordance with claim 1 wherein the poxvirus vector is attenuated.

19. A method in accordance with claim 1 wherein the poxvirus vector is a vaccinia virus vector modified so as to render the virus replication-defective within the treated mammalian host.

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20. A method in accordance with claim 1 wherein the poxvirus vector is an avipoxvirus.

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21. A method in accordance with claim 1 wherein the poxvirus vector is a fowlpoxvirus.

22. A method in accordance with claim 1 wherein the poxvirus vector is MVA.

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23. A method in accordance with claim 1 wherein the poxvirus vector is the NYVAC strain of vaccinia virus.

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24. A method in accordance with claim 1 wherein the poxvirus vector is ALVAC.

30

25. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector of serotype 5 at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant poxvirus vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof; provided said poxvirus vector is replication-impaired in the mammalian host.

5

26. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter

10

(b) inoculating the mammalian host with a boosting immunization comprising a recombinant ALVAC vector comprising a gene encoding the HIV-1 antigen or immunologically relevant modification thereof.

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27. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

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(b) inoculating the mammalian host with a boosting immunization comprising a recombinant ALVAC vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

25

28. A method for inducing an enhanced immunological response against an HIV-1 antigen in a mammalian host, said method comprising the steps of:

(a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 antigen or immunologically relevant modification thereof; and thereafter

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(b) inoculating the mammalian host with a boosting immunization comprising a recombinant MVA vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

5                   29. A method for inducing an enhanced immunological response against an HIV-1 gag antigen in a mammalian host, said method comprising the steps of:

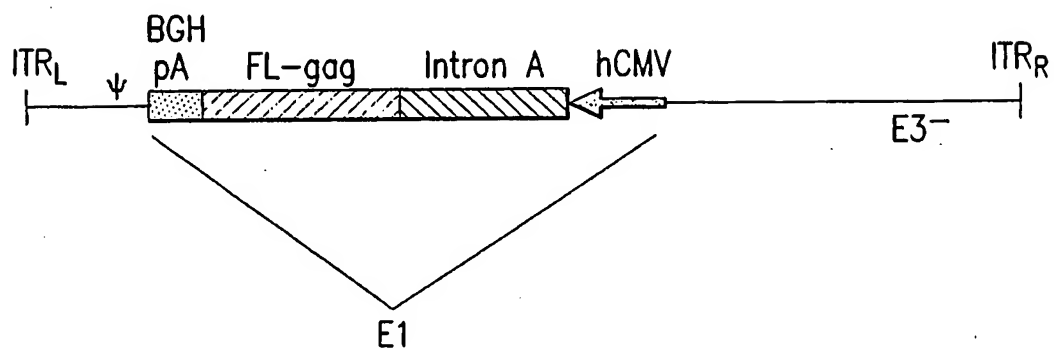
10                   (a) inoculating the mammalian host with a recombinant adenoviral vector at least partially deleted in E1 and devoid of E1 activity comprising a gene encoding an HIV-1 gag antigen or immunologically relevant modification thereof; and thereafter

                  (b) inoculating the mammalian host with a boosting immunization comprising a recombinant MVA vector comprising a gene encoding the HIV-1 gag antigen or immunologically relevant modification thereof.

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ORIGINAL ADENOVECTOR CONSTRUCT:



ORIGINAL HIV-1 gag ADENOVECTOR.

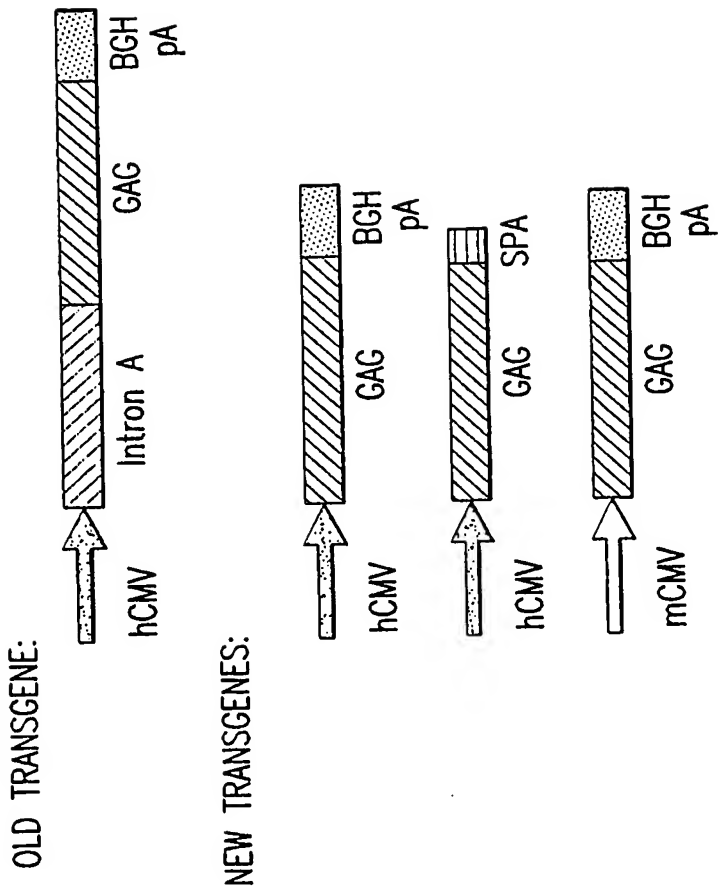
FIG.1

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Sequence of the open reading frame for FL-gag (human codon optimized)

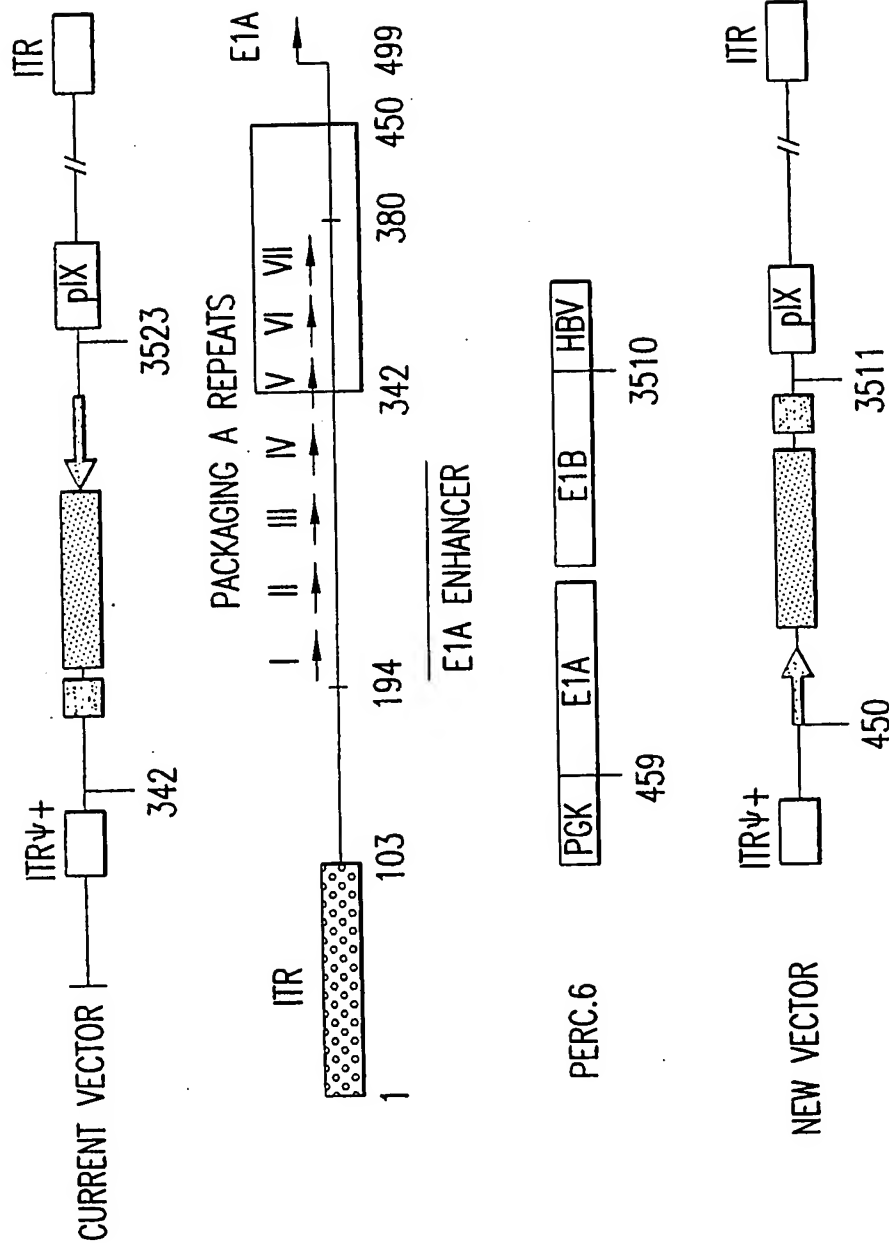
atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtg  
caagaagaagtacaagctaaagcacattgtgtgggcctccaggagctggagaggtttgctgtgaaccctggc  
ctgctggagacctctgaggggtgcaggcagatcctgggccagctccagccctccctgcaaacaggctctgagg  
agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag  
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc  
acaggcaactccagccaggtgtcccagaactacccattgtgcagaacctccagggccagatggtgcaccag  
gccatctccccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctccctgaggtgatccc  
catgttctctgccctgtctgaggggtgccacccccaggacctgaacaccatgctgaacacagtggggggccatc  
aggctgccatgcagatgctgaaggagaccatcaatgaggaggtgctgagtgggacaggctgcatcctgtgc  
acgctggccccattgccccggccagatgagggagcccaggggctctgacattgctggcaccacctccacct  
ccaggagcagattggctggatgaccaacaaccccccatccctgtgggggaaatctacaagaggtggatcat  
cctgggcctgaacaagattgtgaggatgtactccccacctccatcctggacatcaggcagggccccaaggag  
cccttcagggaactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact  
ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggcctg  
ctgccacctggaggagatgatgacagcctgccaggggtggggggccctggtcacaaggccagggtgctg  
gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag  
gaagacagtgaagtgttcaactgtggcaaggtgggccaactgccaagaactgtagggccccaggaaga  
agggtgctggaagtgtggcaaggaggccaccagatgaaggactgcaatgagaggcaggccaacttctg  
ggcaaaatctggccctcccacaagggcaggcctggcaacttctccagtccaggcctgagcccacagccct  
cccgaggagtccttcaggtttggggaggagaagaccacccccagccagaagcaggagcccattgacaagg  
agctgtacccctggcctccctgaggtccctgtttggcaacgaccctcctcccagtaaaataaagcccgggca  
gat

FIG.2



DIAGRAMMATIC REPRESENTATION OF THE ORIGINAL HIV-1 GAG TRANSGENE AND THE SERIES OF NEW TRANSGENE CONSTRUCTIONS.

FIG.3



MODIFICATIONS MADE TO THE CURRENT ADENOVECTOR BACKBONE IN THE GENERATION OF THE NEW VECTOR.

FIG.4



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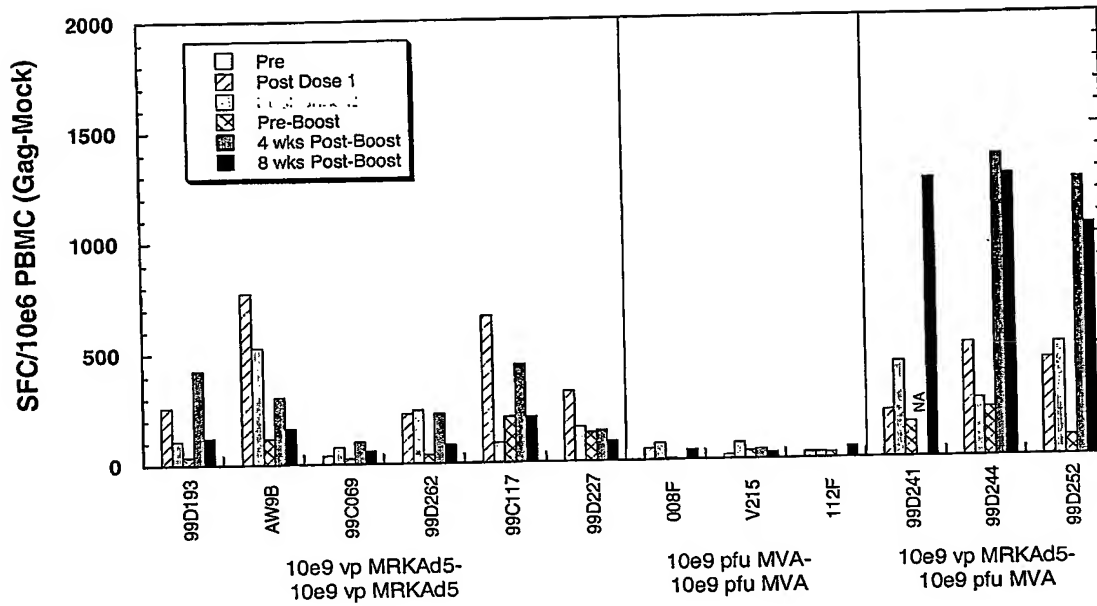


FIG. 5

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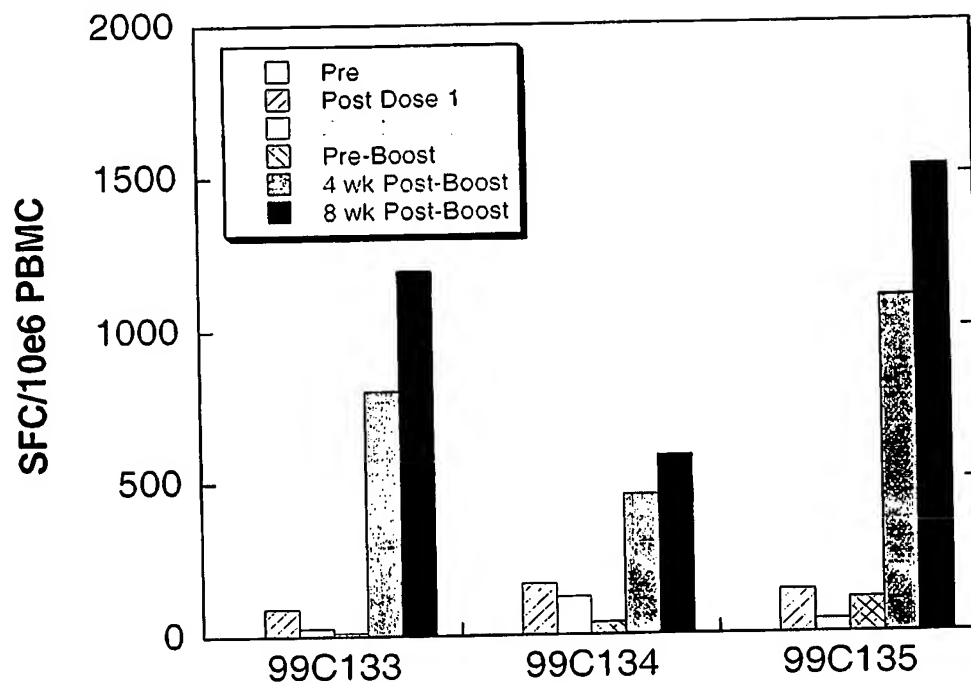
**Ad5-pox Application**

FIG. 6

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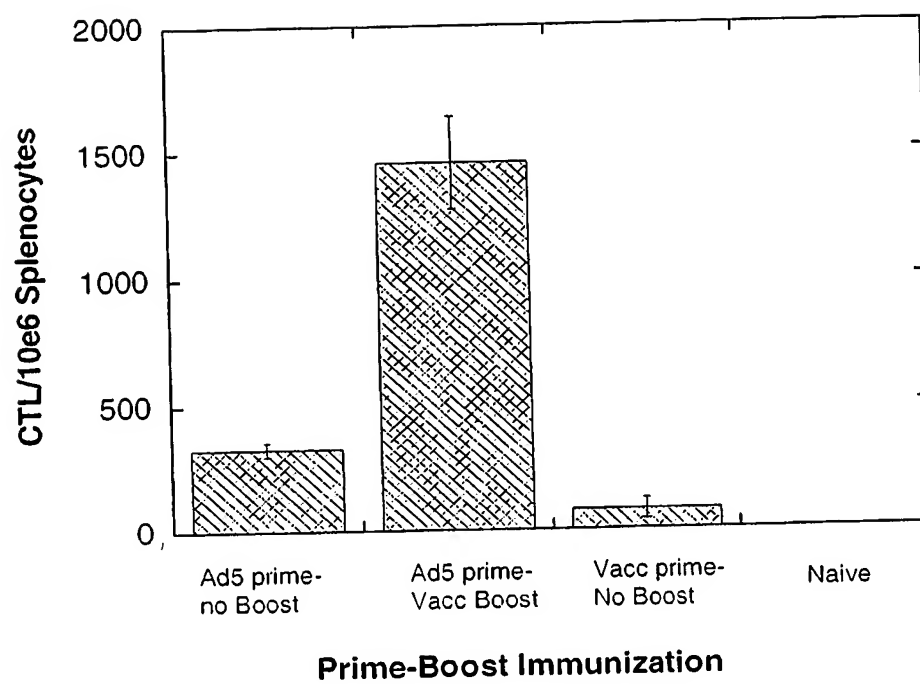


FIG. 7

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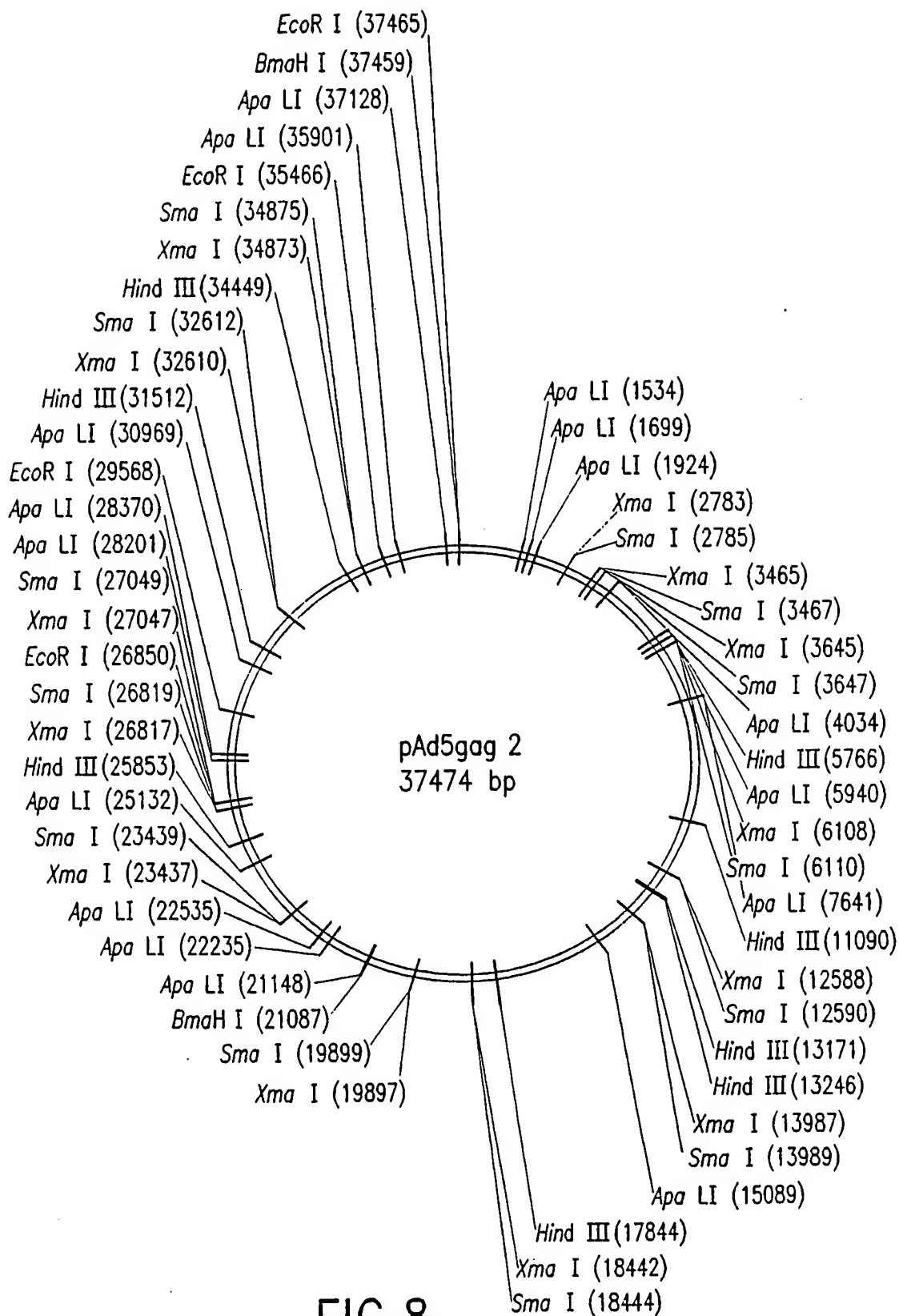


FIG.8

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PacI

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1  TTCTTAATTA ACATCATCAA TAATATACCT TATTTTGGAT TGAAGCCAAT
   AAGAATTAAT TGTAGTAGTT ATTATATGGA ATAAACCTA ACTTCGGTTA

51  ATGATAATGA GGGGGTGGAG TTTGTGACGT GGCGCGGGGC GTGGGAACGG
   TACTATTACT CCCCCACCTC AAACACTGCA CCGCGCCCCG CACCCTTGCC

101  GGCGGGTGAC GTAGTAGTGT GGCAGGAAGTG TGATGTTGCA AGTGTGGCGG
   CCGCCCACTG CATCATCACA CCGCCTTCAC ACTACAACGT TCACACCGCC

151  AACACATGTA AGCGACGGAT GTGGCAAAAG TGACGTTTTT GGTGTGCGCC
   TTGTGTACAT TCGCTGCCTA CACCGTTTTC ACTGCAAAAA CCACACGCGG

201  GGTGTACACA GGAAGTGACA ATTTTCGCGC GGTTTTAGGC GGATGTTGTA
   CCACATGTGT CCTTCACTGT TAAAAGCGCG CCAAATCCG CCTACAACAT

251  GTAAATTTGG GCGTAACCGA GTAAGATTTG GCCATTTTCG CGGGAAACT
   CATTTAAACC CGCATTGGCT CATTCTAAAC CGGTAAAGC GCCCTTTTGA

301  GAATAAGAGG AAGTGAAATC TGAATAATTT TGTGTTACTC ATAGCGCGTA
   CTTATTCTCC TTCACTTTAG ACTTATTAAC ACACAATGAG TATCGCGCAT

351  ATATTTGTCT AGGGCCGCGG GGACTTTGAC CGTTTACGTG GAGACTCGCC
   TATAACAGA TCCCGGCGCC CCTGAAACTG GCAAATGCAC CTCTGAGCGG

401  CAGGTGTTTT TCTCAGGTGT TTTCCGCGTT CCGGGTCAAA GTTGGCGTTT
   GTCCACAAAA AGAGTCCACA AAAGGCGCAA GGCCAGTTT CAACCGCAAA

451  TATTATTATA GCGGGCCGCG ATCCATTGCA TACGTTGTAT CCATATCATA
   ATAATAATAT CCGCCGGCGC TAGGTAACGT ATGCAACATA GGTATAGTAT

501  ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT
   TATACATGTA AATATAACCG AGTACAGGTT GTAATGGCGG TACAACGTGA

551  TGATTATTGA CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA
   ACTAATAACT GATCAATAAT TATCATTAGT TAATGCCCCA GTAATCAAGT

601  TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC
   ATCGGGTATA TACCTCAAGG CGCAATGTAT TGAATGCCAT TTACCGGGCG

651  CTGGCTGACC GCCCAACGAC CCCC GCCCAT TGACGTCAAT AATGACGTAT
   GACCGACTGG CGGGTTGCTG GGGCGGGTA ACTGCAGTTA TTAGTGCATA

701  GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA
   CAAGGGTATC ATTGCGGTGA TCCCTGAAAG GTAAGTGCAG TTACCCACCT

751  GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC
   CATAAATGCC ATTTGACGGG TGAACCGTCA TGTAGTTCAC ATAGTATACG

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FIG.9A-1

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801 CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT  
GTTTCATGCGG GGGATAACTG CAGTTACTGC CATTTACCGG GCGGACCGTA

851 TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA  
ATACGGGTCA TGTACTGGAA TACCCTGAAA GGATGAACCG TCATGTAGAT

901 CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA  
GCATAATCAG TAGCGATAAT GGTACCACTA CGCCAAAACC GTCATGTAGT

951 ATGGGCGTGG ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC  
TACCCGCACC TATCGCCAAA CTGAGTGCCC CTAAAGGTTC AGAGGTGGGG

1001 ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC GGGACTTTCC  
TAACTGCAGT TACCCTCAAA CAAAACCGTG GTTTTAGTTG CCCTGAAAGG

1051 AAAATGTCGT AACAACTCCG CCCATTGAC GCAAATGGGC GGTAGGCGTG  
TTTTACAGCA TTGTTGAGGC GGGGTAAC TG TTTTACCG CCATCCGCAC

1101 TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC  
ATGCCACCCT CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG

1151 GCCTGGAGAC GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA  
CGGACCTCTG CGGTAGGTGC GACAAAAC TG TTTTACCG CTGTGGCCCT

1201 CCGATCCAGC CTCCGCGGCC GGGAACGGTG CATTGGAACG CGGATTCCCC  
GGCTAGGTCG GAGGCGCCGG CCCTTGCCAC GTAACTTGC GCCTAAGGGG

1251 GTGCCAAGAG TGAGATCTAC CATGGGTGCT AGGGCTTCTG TGCTGTCTGG  
CACGGTTCTC ACTCTAGATG GTACCCACGA TCCCGAAGAC ACGACAGACC

1301 TGGTGAGCTG GACAAGTGGG AGAAGATCAG GCTGAGGCCT GGTGGCAAGA  
ACCACTCGAC CTGTTACCC TCTTCTAGTC CGACTCCGGA CCACCGTTCT

1351 AGAAGTACAA GCTAAAGCAC ATTGTGTGGG CCTCCAGGGA GCTGGAGAGG  
TCTTCATGTT CGATTTCTG TAACACACCC GGAGGTCCCT CGACCTCTCC

1401 TTTGCTGTGA ACCCTGGCCT GCTGGAGACC TCTGAGGGGT GCAGGCAGAT  
AAACGACACT TGGGACCGGA CGACCTCTGG AGACTCCCCA CGTCCGTCTA

1451 CCTGGGCCAG CTCCAGCCCT CCCTGCAAAC AGGCTCTGAG GAGCTGAGGT  
GGACCCGGTC GAGGTCGGGA GGGACGTTTG TCCGAGACTC CTCGACTCCA

1501 CCCTGTACAA CACAGTGGCT ACCCTGTACT GTGTGCACCA GAAGATTGAT  
GGGACATGTT GTGTCACCGA TGGGACATGA CACACGTGGT CTTCTAACTA

1551 GTGAAGGACA CCAAGGAGGC CCTGGAGAAG ATTGAGGAGG AGCAGAACAA  
CACTTCCTGT GGTTCTCTCC GGACCTCTC TAACTCCTCC TCGTCTTGTT

1601 GTCCAAGAAG AAGGCCCAGC AGGCTGCTGC TGGCACAGGC AACTCCAGCC  
CAGGTTCTTC TTCCGGGTCG TCCGACGACG ACCGTGTCCG TTGAGGTCGG

FIG.9A-2

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1651 AGGTGTCCCA GAACTACCCC ATTGTGCAGA ACCTCCAGGG CCAGATGGTG  
 TCCACAGGGT CTTGATGGGG TAACACGTCT TGGAGGTCCC GGTCTACCAC  
 1701 CACCAGGCCA TCTCCCCCG GACCCTGAAT GCCTGGGTGA AGGTGGTGGA  
 GTGGTCCGGT AGAGGGGGGC CTGGGACTTA CGGACCCACT TCCACCACCT  
 1751 GGAGAAGGCC TTCTCCCCTG AGGTGATCCC CATGTTCTCT GCCCTGTCTG  
 CCTCTTCCGG AAGAGGGGAC TCCACTAGGG GTACAAGAGA CGGGACAGAC  
 1801 AGGGTGCCAC CCCCAGGAC CTGAACACCA TGCTGAACAC AGTGGGGGGC  
 TCCCACGGTG GGGGGTCCTG GACTTGTGGT ACGACTTGTG TCACCCCCCG  
 1851 CATCAGGCTG CCATGCAGAT GCTGAAGGAG ACCATCAATG AGGAGGCTGC  
 GTAGTCCGAC GGTACGTCTA CGACTTCCTC TGGTAGTTAC TCCTCCGACG  
 1901 TGAGTGGGAC AGGCTGCATC CTGTGCACGC TGGCCCCATT GCCCCGGCC  
 ACTCACCCTG TCCGACGTAG GACACGTGCG ACCGGGGTAA CGGGGGCCGG  
 1951 AGATGAGGGA GCCCAGGGGC TCTGACATTG CTGGCACCAC CTCCACCCTC  
 TCTACTCCCT CGGGTCCCCG AGACTGTAAC GACCGTGGTG GAGGTGGGAG  
 2001 CAGGAGCAGA TTGGCTGGAT GACCAACAAC CCCCCATCC CTGTGGGGGA  
 GTCCTCGTCT AACCGACCTA CTGGTTGTTG GGGGGGTAGG GACACCCCT  
 2051 AATCTACAAG AGGTGGATCA TCCTGGGCCT GAACAAGATT GTGAGGATGT  
 TTAGATGTTT TCCACCTAGT AGGACCCGGA CTTGTTCTAA CACTCTACA  
 2101 ACTCCCCAC CTCCATCCTG GACATCAGGC AGGGCCCCAA GGAGCCCTTC  
 TGAGGGGGTG GAGGTAGGAC CTGTAGTCCG TCCCGGGGTT CCTCGGGAAG  
 2151 AGGGACTATG TGGACAGGTT CTACAAGACC CTGAGGGCTG AGCAGGCCTC  
 TCCCTGATAC ACCTGTCCAA GATGTTCTGG GACTCCCGAC TCGTCCGGAG  
 2201 CCAGGAGGTG AAGAACTGGA TGACAGAGAC CCTGCTGGTG CAGAATGCCA  
 GGTCTCTCAC TTCTTGACCT ACTGTCTCTG GGACGACCAC GTCTTACGGT  
 2251 ACCCTGACTG CAAGACCATC CTGAAGGCCC TGGGCCCTGC TGCCACCCTG  
 TGGGACTGAC GTTCTGGTAG GACTTCCGGG ACCCGGGACG ACGGTGGGAC  
 2301 GAGGAGATGA TGACAGCCTG CCAGGGGGTG GGGGGCCCTG GTCACAAGGC  
 CTCCTCTACT ACTGTCGGAC GGTCCCCAC CCCCCGGGAC CAGTGTTCCG  
 2351 CAGGGTGCTG GCTGAGGCCA TGTCCCAGGT GACCAACTCC GCCACCATCA  
 GTCCCACGAC CGACTCCGGT ACAGGGTCCA CTGGTTGAGG CGGTGGTAGT  
 2401 TGATGCAGAG GGGCAACTTC AGGAACCAGA GGAAGACAGT GAAGTGCTTC  
 ACTACGTCTC CCCGTTGAAG TCCTTGGTCT CTTCTGTCA CTTACGAAG  
 2451 AACTGTGGCA AGGTGGGCCA CATTGCCAAG AACTGTAGGG CCCCAGGAA  
 TTGACACCGT TCCACCCGGT GTAACGGTTC TTGACATCCC GGGGGTCCTT

FIG.9A-3

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2501 GAAGGGCTGC TGGAAAGTGTG GCAAGGAGGG CCACCAGATG AAGGACTGCA  
CTTCCCAGAC ACCTTCACAC CGTTCCTCCC GGTGGTCTAC TTCCTGACGT

2551 ATGAGAGGCA GGCCAACTTC CTGGGCAAAA TCTGGCCCTC CCACAAGGGC  
TACTCTCCGT CCGGTTGAAG GACCCGTTTT AGACCGGGAG GGTGTTCCCC

2601 AGGCCTGGCA ACTTCCTCCA GTCCAGGCCT GAGCCCACAG CCCCTCCCGA  
TCCGGACCGT TGAAGGAGGT CAGGTCCGGA CTCGGGTGTC GGGGAGGGCT

2651 GGAGTCCTTC AGGTTTGGGG AGGAGAAGAC CACCCCAGC CAGAAGCAGG  
CCTCAGGAAG TCCAAACCCC TCCTCTTCTG GTGGGGGTCT GTCTTCGTCC

2701 AGCCCATTTGA CAAGGAGCTG TACCCCTGG CCTCCCTGAG GTCCCTGTTT  
TCGGGTAACT GTTCCTCGAC ATGGGGGACC GGAGGGACTC CAGGGACAAA

2751 GGCAACGACC CCTCCTCCCA GTAAAATAAA GCCCGGGCAG ATCTGCTGTG  
CCGTTGCTGG GGAGGAGGGT CATTTTATTT CGGGCCCGTC TAGACGACAC

2801 CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT  
GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC ACGGAAGGAA

2851 GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA  
CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT TTA CTCTTT

2901 TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG  
AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC CCCACCCAC

2951 GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG  
CCCGTCCTGT CGTTCCCCCT CCTAACCTT CTGTTATCGT CCGTACGACC

3001 GGATGCGGTG GGCTCTATGG CCGATCGGCG CGCCGTA CTG AAATGTGTGG  
CCTACGCCAC CCGAGATACC GGCTAGCCGC GCGGCATGAC TTTACACACC

3051 GCGTGGCTTA AGGGTGGGAA AGAATATATA AGGTGGGGGT CTTATGTAGT  
CGCACCGAAT TCCCACCCTT TCTTATATAT TCCACCCCA GAATACATCA

3101 TTTGTATCTG TTTTGCAGCA GCCGCCGCCG CCATGAGCAC CAACTCGTTT  
AAACATAGAC AAAACGTCGT CGGCGGCGGC GGTACTCGTG GTTGAGCAAA

3151 GATGGAAGCA TTGTGAGCTC ATATTTGACA ACGCGCATGC CCCCATGGGC  
CTACCTTCGT AACACTCGAG TATAAACTGT TGCGCGTACG GGGGTACCCG

3201 CGGGGTGCGT CAGAATGTGA TGGGCTCCAG CATTGATGGT CGCCCCGTCC  
GCCCCACGCA GTCTTACACT ACCCGAGGTC GTA ACTACCA GCGGGGACAG

3251 TGCCCGCAAA CTCTACTACC TTGACCTACG AGACCGTGTC TGGAACGCCG  
ACGGGCGTTT GAGATGATGG AACTGGATGC TCTGGCACAG ACCTTGCGGC

3301 TTGGAGACTG CAGCCTCCGC CGCCGCTTCA GCCGCTGCAG CCACCGCCCC  
AACCTCTGAC GTCGGAGGCG GCGGCGAAGT CGGCGACGTC GGTGGCGGGC

FIG.9A-4



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3351 CGGGATTGTG ACTGACTTTG CTTTCCTGAG CCCGCTTGCA AACAGTGCAG  
 GCCCTAACAC TGACTGAAAC GAAAGGACTC GGGCGAACGT TTGTCACGTC  
 3401 CTTCCCGTTC ATCCGCCCCG GATGACAAGT TGACGGCTCT TTTGGCACAA  
 GAAGGGCAAG TAGGCGGGCG CTA CTGTTCA ACTGCCGAGA AAACCGTGTT  
 3451 TTGGATTCTT TGACCCGGGA ACTTAATGTC GTTTCTCAGC AGCTGTTGGA  
 AACCTAAGAA ACTGGGCCCT TGAATTACAG CAAAGAGTCG TCGACAACCT  
 3501 TCTGCGCCAG CAGGTTTCTG CCCTGAAGGC TTCCTCCCCT CCCAATGCGG  
 AGACGCGGTC GTCCAAAGAC GGGACTTCCG AAGGAGGGGA GGGTTACGCC  
 3551 TTTAAACAT AAATAAAAA CCAGACTCTG TTTGGATTG GATCAAGCAA  
 AAATTTTGTA TTTATTTTTT GGTCTGAGAC AAACCTAAAC CTAGTTCGTT  
 3601 GTGTCTTGCT GTCTTTATTT AGGGGTTTTG CGCGCGCGGT AGGCCCGGGA  
 CACAGAACGA CAGAAATAAA TCCCCAAAAC GCGCGCGCCA TCCGGGCCCT  
 3651 CCAGCGGTCT CGGTCGTTGA GGGTCCTGTG TATTTTTTCC AGGACGTGGT  
 GGTGCGCAGA GCCAGCAACT CCCAGGACAC ATAAAAAGG TCCTGCACCA  
 3701 AAAGGTGACT CTGGATGTTT AGATACATGG GCATAAGCCC GTCTCTGGGG  
 TTTCCACTGA GACCTACAAG TCTATGTACC CGTATTCGGG CAGAGACCCC  
 3751 TGGAGGTAGC ACCACTGCAG AGCTTCATGC TCGGGGGTGG TGTTGTAGAT  
 ACCTCCATCG TGGTGACGTC TCGAAGTACG ACGCCCCACC ACAACATCTA  
 3801 GATCCAGTCG TAGCAGGAGC GCTGGGCGTG GTGCCTAAAA ATGTCTTTCA  
 CTAGGTCAGC ATCGTCCTCG CGACCCGCAC CACGGATTTT TACAGAAAGT  
 3851 GTAGCAAGCT GATTGCCAGG GGCAGGCCCT TGGTGTAAGT GTTTACAAAG  
 CATCGTTTCA CTAACGGTCC CCGTCCGGGA ACCACATTCA CAAATGTTTC  
 3901 CGGTTAAGCT GGGATGGGTG CACATCGTGGG GATATGAGAT GCATCTTGGA  
 GCCAATTCTA CCCTACCCAC GTATGCACCC CTATACTCTA CGTAGAACCT  
 3951 CTGTATTTTT AGGTTGGCTA TGTTCCCAGC CATATCCCTC CGGGGATTCA  
 GACATAAAAA TCCAACCGAT ACAAGGGTCG GTATAGGGAG GCCCCTAAGT  
 4001 TGTTGTGCAG AACCACCAGC ACAGTGTATC CGGTGCACTT GGGAAATTTG  
 ACAACACGTC TTGGTGGTCG TGTCACATAG GCCACGTGAA CCTTTTAAAC  
 4051 TCATGTAGCT TAGAAGGAAA TCGTGGAAG AACTTGGAGA CGCCCTTGTTG  
 AGTACATCGA ATCTTCCTTT ACGCACCTTC TTGAACCTCT GCGGGAACAC  
 4101 ACCTCCAAGA TTTTCCATGC ATTCGTCCAT AATGATGGCA ATGGGCCCAC  
 TGGAGGTTCT AAAAGGTACG TAAGCAGGTA TTA CTACCGT TACCCGGGTG  
 4151 GGGCGGCGGC CTGGGCGAAG ATATTTCTGG GATCACTAAC GTCATAGTTG  
 CCCGCCGCCG GACCCGCTTC TATAAAGACC CTAGTGATTG CAGTATCAAC

FIG.9A-5

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4201 TGTTCCAGGA TGAGATCGTC ATAGGCCATT TTTACAAAGC GCGGGCGGAG  
ACAAGGTCCT ACTCTAGCAG TATCCGGTAA AAATGTTTCG CGCCCGCCTC

4251 GGTGCCAGAC TGCGGTATAA TGGTTCCATC CGGCCAGGG GCGTAGTTAC  
CCACGGTCTG ACGCCATATT ACCAAGGTAG GCCGGGTCCC CGCATCAATG

4301 CCTCACAGAT TTGCATTTCC CACGCTTTGA GTTCAGATGG GGGGATCATG  
GGAGTGTCTA AACGTAAAGG GTGCGAAACT CAAGTCTACC CCCCTAGTAC

4351 TCTACCTGCG GGGCGATGAA GAAAACGGTT TCCGGGGTAG GGGAGATCAG  
AGATGGACGC CCCGCTACTT CTTTGTCCAA AGGCCCATC CCCTCTAGTC

4401 CTGGGAAGAA AGCAGGTTCC TGAGCAGCTG CGACTTACCG CAGCCGGTGG  
GACCTTCTT TCGTCCAAGG ACTCGTCGAC GCTGAATGGC GTCGGCCACC

4451 GCCCGTAAAT CACACCTATT ACCGGCTGCA ACTGGTAGTT AAGAGAGCTG  
CGGGCATTTA GTGTGGATAA TGGCCGACGT TGACCATCAA TTCTCTCGAC

4501 CAGCTGCCGT CATCCCTGAG CAGGGGGGCC ACTTCGTAA GCATGTCCCT  
GTCGACGGCA GTAGGGACTC GTCCCCCGG TGAAGCAATT CGTACAGGGA

4551 GACTCGCATG TTTTCCCTGA CCAAATCCGC CAGAAGGCGC TCGCCGCCCA  
CTGAGCGTAC AAAAGGGACT GGTTTAGGCG GTCTTCCGCG AGCGGCGGGT

4601 GCGATAGCAG TTCTTGCAAG GAAGCAAAGT TTTTCAACGG TTTGAGACCG  
CGCTATCGTC AAGAACGTTT CTTCTGTTCA AAAAGTTGCC AACTCTGGC

4651 TCCGCCGTAG GCATGCTTTT GAGCGTTTGA CCAAGCAGTT CCAGGCGGTC  
AGGCGGCATC CGTACGAAAA CTCGCAAACT GGTTCTGCAA GGTCCGCCAG

4701 CCACAGCTCG GTCACCTGCT CTACGGCATC TCGATCCAGC ATATCTCCTC  
GGTGTGAGC CAGTGGACGA GATGCCGTAG AGCTAGGTG TATAGAGGAG

4751 GTTTCGCGGG TTGGGGCGGC TTTGCTGTA CGGCAGTAGT CCGTGCTCGT  
CAAAGCGCCC AACCCGCGC AAAGCGACAT GCCGTCATCA GCCACGAGCA

4801 CCAGACGGGC CAGGGTCATG TCTTTCCACG GGCAGGGT CCTCGTCAGC  
GGTCTGCCCC GTCCAGTAC AGAAAGGTGC CCGCTGCCA GGAGCAGTCG

4851 GTAGTCTGGG TCACGGTGAA GGGGTGCGCT CCGGGCTGCG CGCTGGCCAG  
CATCAGACCC AGTGCCACTT CCCCACGCGA GGCCCGACGC GCGACCGGTC

4901 GGTGCGCTTG AGGCTGGTCC TGCTGGTGCT GAAGCGCTGC CCGTCTTCGC  
CCACGCGAAC TCCGACCAGG ACGACCACGA CTTGCGGACG GCCAGAAGCG

4951 CCTGCGCGTC GGCCAGGTAG CATTTGACCA TGGTGTCATA GTCCAGCCCC  
GGACGCGCAG CCGGTCCATC GTAAACTGGT ACCACAGTAT CAGGTCGGGG

5001 TCCGCGGCGT GGCCCTTGGC GCGCAGCTTG CCCTTGGAGG AGGCGCCGCA  
AGGCGCCGCA CCGGGAACCG CGCGTCGAAC GGGAACCTCC TCCGCGGCGT

FIG.9A-6

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5051 CGAGGGGCAG TGCAGACTTT TGAGGGCGTA GAGCTTGGGC GCGAGAAATA  
GCTCCCCGTC ACGTCTGAAA ACTCCCGCAT CTCGAACCCG CGCTCTTTAT

5101 CCGATTCCGG GGAGTAGGCA TCCGCGCCGC AGGCCCCGCA GACGGTCTCG  
GGCTAAGGCC CCTCATCCGT AGGCGCGGCG TCCGGGGCGT CTGCCAGAGC

5151 CATTCCACGA GCCAGGTGAG CTCTGGCCGT TCGGGGTCAA AAACCAGGTT  
GTAAGGTGCT CGGTCCACTC GAGACCGGCA AGCCCCAGTT TTTGGTCCAA

5201 TCCCCCATGC TTTTGTATGC GTTCTTACC TCTGGTTTCC ATGAGCCGGT  
AGGGGGTACG AAAAATACTAG CAAAGAATGG AGACCAAAGG TACTCGGCCA

5251 GTCCACGCTC GGTGACGAAA AGGCTGTCCG TGTCCCCGTA TACAGACTTG  
CAGGTGCGAG CCACTGCTTT TCCGACAGGC ACAGGGGCAT ATGTCTGAAC

5301 AGAGGCCTGT CCTCGAGCGG TGTTCCGCGG TCCTCCTCGT ATAGAACTC  
TCTCCGGACA GGAGCTCGCC ACAAGGCGCC AGGAGGAGCA TATCTTTGAG

5351 GGACCACTCT GAGACAAAGG CTCGCGTCCA GGCCAGCACG AAGGAGGCTA  
CCTGGTGAGA CTCTGTTTCC GAGCGCAGGT CCGGTCGTGC TTCCTCCGAT

5401 AGTGGGAGGG GTAGCGGTCTG TTGTCCACTA GGGGGTCCAC TCGCTCCAGG  
TCACCCTCCC CATCGCCAGC AACAGGTGAT CCCCCAGGTG AGCGAGGTCC

5451 GTGTGAAGAC ACATGTCGCC CTCTTCGGCA TCAAGGAAGG TGATTGGTTT  
CACACTTCTG TGTACAGCGG GAGAAGCCGT AGTTCCTTCC ACTAACCAAA

5501 GTAGGTGTAG GCCACGTGAC CGGGTGTTC TGAAGGGGGG CTATAAAAGG  
CATCCACATC CGGTGCACTG GCCCACAAGG ACTTCCCCC GATATTTTCC

5551 GGGTGGGGGC GCGTTCGTCC TCACTCTCTT CCGCATCGCT GTCTGCGAGG  
CCCACCCCCG CGCAAGCAGG AGTGAGAGAA GGCGTAGCGA CAGACGCTCC

5601 GCCAGCTGTT GGGGTGAGTA CTCCCTCTGA AAAGCGGGCA TGAATTCTGC  
CGGTCGACAA CCCCCTCAT GAGGGAGACT TTTCGCCCGT ACTGAAGACG

5651 GCTAAGATTG TCAGTTTCCA AAAACGAGGA GGATTTGATA TTCACCTGGC  
CGATTCTAAC AGTCAAAGGT TTTTGCTCCT CTAAACTAT AAGTGGACCG

5701 CCGCGGTGAT GCCTTTGAGG GTGGCCGCAT CCATCTGGTC AGAAAAGACA  
GGCGCCACTA CGGAAACTCC CACCGGCGTA GGTAGACCAG TCTTTTCTGT

5751 ATCTTTTTGT TGTCAAGCTT GGTGGCAAAC GACCCGTAGA GGGCGTTGGA  
TAGAAAAACA ACAGTTCGAA CCACCGTTTG CTGGGCATCT CCCGCAACCT

5801 CAGCAACTTG GCGATGGAGC GCAGGGTTTG GTTTTTGTCT CGATCGGCGC  
GTCGTTGAAC CGCTACCTCG CGTCCCAAAC CAAAAACAGC GCTAGCCGCG

5851 GCTCCTTGGC CGCGATGTTT AGCTGCACGT ATTCGCGCGC AACGCACCGC  
CGAGGAACCG GCGCTACAAA TCGACGTGCA TAAGCGCGCG TTGCGTGGCG

FIG.9A-7

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5901 CATTCCGGAA AGACGGTGGT GCGCTCGTCG GGCACCAGGT GCACGCGCCA  
GTAAGCCCTT TCTGCCACCA CGCGAGCAGC CCGTGGTCCA CGTGCGCGGT

5951 ACCGCGGTTG TGCAGGGTGA CAAGGTCAAC GCTGGTGGCT ACCTCTCCGC  
TGGCGCCAAC ACGTCCCACT GTTCCAGTTG CGACCACCGA TGGAGAGGCG

6001 GTAGGCGCTC GTTGGTCCAG CAGAGGCGGC CGCCCTTGCG CGAGCAGAAT  
CATCCGCGAG CAACCAGGTC GTCTCCGCCG GCGGGAACGC GCTCGTCTTA

6051 GGCGGTAGGG GGTCTAGCTG CGTCTCGTCC GGGGGGTCTG CGTCCACGGT  
CCGCCATCCC CCAGATCGAC GCAGAGCAGG CCCCCAGAC GCAGGTGCCA

6101 AAAGACCCCG GGCAGCAGGC GCGCGTCGAA GTAGTCTATC TTGCATCCTT  
TTTCTGGGGC CCGTCGTCCG CGCGCAGCTT CATCAGATAG AACGTAGGAA

6151 GCAAGTCTAG CGCCTGCTGC CATGCGCGGG CGGCAAGCGC GCGCTCGTAT  
CGTTCAGATC GCGGACGACG GTACGCGCCC GCCGTTGCGG CGCGAGCATA

6201 GGGTTGAGTG GGGGACCCCA TGGCATGGGG TGGGTGAGCG CGGAGGCGTA  
CCCAACTCAC CCCCTGGGGT ACCGTACCCC ACCCACTCGC GCCTCCGCAT

6251 CATGCCGCAA ATGTCGTAAA CGTAGAGGGG CTCTCTGAGT ATTCCAAGAT  
GTACGGCGTT TACAGCATTT GCATCTCCCC GAGAGACTCA TAAGTTCTA

6301 ATGTAGGGTA GCATCTTCCA CCGCGGATGC TGGCGCGCAC GTAATCGTAT  
TACATCCCAT CGTAGAAGGT GGCGCCTACG ACCGCGCGTG CATTAGCATA

6351 AGTTCGTGCG AGGGAGCGAG GAGGTCGGGA CCGAGGTTGC TACGGGCGGG  
TCAAGCACGC TCCCTCGCTC CTCCAGCCCT GGCTCCAACG ATGCCCGCCC

6401 CTGCTCTGCT CGGAAGACTA TCTGCCTGAA GATGGCATGT GAGTTGGATG  
GACGAGACGA GCCTTCTGAT AGACGGACTT CTACCGTACA CTCAACCTAC

6451 ATATGGTTGG ACGCTGGAAG ACGTTGAAGC TGGCGTCTGT GAGACCTACC  
TATACCAACC TGCGACCTTC TGCAACTTCG ACCGCAGACA CTCTGGATGG

6501 GCGTCACGCA CGAAGGAGGC GTAGGAGTCG CGCAGCTTGT TGACCAGCTC  
CGCAGTGCGT GCTTCCTCCG CATCCTCAGC GCGTCGAACA ACTGGTCGAG

6551 GGCGGTGACC TGCACGTCTA GGGCGCAGTA GTCCAGGGTT TCCTTGATGA  
CCGCCACTGG ACGTGCAGAT CCCGCGTCAT CAGGTCCCAA AGGAACTACT

6601 TGTCATACTT ATCCTGTCCC TTTTTTTTCC ACAGCTCGCG GTTGAGGACA  
ACAGTATGAA TAGGACAGGG AAAAAAAGG TGTCGAGCGC CAACTCCTGT

6651 AACTCTTCGC GGTCTTTCCA GTA CTCTTG G ATCGGAAACC CGTCGGCCTC  
TTGAGAAGCG CCAGAAAGGT CATGAGAACC TAGCCTTTGG GCAGCCGGAG

6701 CGAACGGTAA GAGCCTAGCA TG TAGAACTG GTTGACGGCC TGGTAGGCGC  
GCTTGCCATT CTCGGATCGT ACATCTTGAC CAACTGCCGG ACCATCCGCG

FIG.9A-8

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6751 AGCATCCCTT TTCTACGGGT AGCGCGTATG CCTGCGCGGC CTTCCGGAGC  
TCGTAGGGAA AAGATGCCCA TCGCGCATAC GGACGCGCCG GAAGGCCTCG

6801 GAGGTGTGGG TGAGCGCAAA GGTGTCCCTG ACCATGACTT TGAGGTACTG  
CTCCACACCC ACTCGCGTTT CCACAGGGAC TGGTACTGAA ACTCCATGAC

6851 GTATTTGAAG TCAGTGTCTG CGCATCCGCC CTGCTCCCAG AGCAAAAAGT  
CATAAACTTC AGTCACAGCA GCGTAGGCGG GACGAGGGTC TCGTTTTTCA

6901 CCGTGCGCTT TTTGGAACGC GGATTTGGCA GGGCGAAGGT GACATCGTTG  
GGCACGCGAA AAACCTTGCG CCTAAACCGT CCCGCTTCCA CTGTAGCAAC

6951 AAGAGTATCT TTCCCGCGCG AGGCATAAAG TTGCGTGTGA TGCGBAAGGG  
TTCTCATAGA AAGGGCGCGC TCCGTATTTC AACGCACACT ACGCCTTCCC

7001 TCCCGGCACC TCGGAACGGT TGTTAATTAC CTGGGCGGCG AGCAGCATCT  
AGGGCCGTGG AGCCTTGCCA ACAATTAATG GACCCGCCGC TCGTGCTAGA

7051 CGTCAAAGCC GTTGATGTTG TGGCCACAA TGTAAGTTC CAAGAAGCGC  
GCAGTTTCGG CAACTACAAC ACCGGGTGTT ACATTTCAAG GTTCTTCGCG

7101 GGGATGCCCT TGATGGAAGG CAATTTTTTA AGTTCCTCGT AGGTGAGCTC  
CCCTACGGGA ACTACCTTCC GTTAAAAAAT TCAAGGAGCA TCCACTCGAG

7151 TTCAGGGGAG CTGAGCCCGT GCTCTGAAAG GGCCAGTCT GCAAGATGAG  
AAGTCCCCTC GACTCGGGCA CGAGACTTTC CCGGGTCAGA CGTTCTACTC

7201 GGTTGGAAGC GACGAATGAG CTCCACAGGT CACGGGCCAT TAGCATTTGC  
CCAACCTTCG CTGCTTACTC GAGGTGTCCA GTGCCCGGTA ATCGTAAACG

7251 AGGTGGTCGC GAAAGGTCCT AAAGTGGCGA CCTATGGCCA TTTTTTCTGG  
TCCACCAGCG CTTTCCAGGA TTTGACCGCT GGATACCGGT AAAAAAGACC

7301 GGTGATGCAG TAGAAGGTAA GCGGGTCTTG TTCCAGCGG TCCCATCCAA  
CCACTACGTC ATCTTCCATT CGCCCAGAAC AAGGGTCGCC AGGGTAGGTT

7351 GGTTGCGCGC TAGGTCTCGC GCGGCAGTCA CTAGAGGCTC ATCTCCGCCG  
CCAAGCGCCG ATCCAGAGCG CGCCGTCAGT GATCTCCGAG TAGAGGCGGC

7401 AACTTCATGA CCAGCATGAA GGGCACGAGC TGCTTCCCAA AGGCCCCCAT  
TTGAAGTACT GGTGCTACTT CCCGTGCTCG ACGAAGGGTT TCCGGGGGTA

7451 CCAAGTATAG GTCTCTACAT CGTAGGTGAC AAAGAGACGC TCGGTGCGAG  
GGTTCATATC CAGAGATGTA GCATCCACTG TTTCTCTGCG AGCCACGCTC

7501 GATGCGAGCC GATCGGGAAG AACTGGATCT CCCGCCACCA ATTGGAGGAG  
CTACGCTCGG CTAGCCCTTC TTGACCTAGA GGGCGGTGGT TAACCTCCTC

7551 TGGCTATTGA TGTGGTGAAA GTAGAAGTCC CTGCGACGGG CCGAACACTC  
ACCGATAACT ACACCACTTT CATCTTCAGG GACGCTGCCG GGCTTGTGAG

FIG.9A-9

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7601 GTGCTGGCTT TTGTAAAAAC GTGCGCAGTA CTGGCAGCGG TGCACGGGCT  
CACGACCGAA AACATTTTTG CACGCGTCAT GACCGTCGCC ACGTGCCCGA

7651 GTACATCCTG CACGAGGTTG ACCTGACGAC CGCGCACAAG GAAGCAGAGT  
CATGTAGGAC GTGCTCCAAC TGGACTGCTG GCGCGTGTTT CTTCGTCTCA

7701 GGGAATTTGA GCCCCTCGCC TGGCGGGTTT GGCTGGTGGT CTTCTACTTC  
CCCTTAAACT CGGGGAGCGG ACCGCCCAA CCGACCACCA GAAGATGAAG

7751 GGCTGCTTGT CTTGACCGT CTGGCTGCTC GAGGGGAGTT ACGGTGGATC  
CCGACGAACA GGAAGTGGCA GACCGACGAG CTCCCCTCAA TGCCACCTAG

7801 GGACCACCAC GCCGCGCGAG CCCAAAGTCC AGATGTCCGC GCGCGGCGGT  
CCTGGTGGTG CGGCGCGCTC GGGTTTCAGG TCTACAGGCG CGCGCCGCCA

7851 CGGAGCTTGA TGACAACATC GCGCAGATGG GAGCTGTCCA TGGTCTGGAG  
GCCTCGAACT ACTGTTGTAG CGCGTCTACC CTCGACAGGT ACCAGACCTC

7901 CTCCCGCGGC GTCAGGTCAG GCGGGAGCTC CTGCAGGTTT ACCTCGCATA  
GAGGGCGCCG CAGTCCAGTC CGCCCTCGAG GACGTCCAA TGGAGCGTAT

7951 GACGGGTCAG GCGCGGGGCT AGATCCAGGT GATACCTAAT TTCCAGGGGC  
CTGCCAGTC CCGCGCCCGA TCTAGGTCCA CTATGGATTA AAGGTCCCCG

8001 TGGTTGGTGG CGGCGTCGAT GGCTTGCAAG AGGCCGCATC CCCGCGGCGC  
ACCAACCACC GCCGCAGCTA CCGAACGTTT TCCGGCGTAG GGGCGCCGCG

8051 GACTACGGTA CCGCGCGGCG GCGGGTGGGC CGCGGGGGTG TCCTTGATG  
CTGATGCCAT GCGCGGCCG CCGCCACCCG GCGCCCCAC AGGAACCTAC

8101 ATGCATCTAA AAGCGGTGAC GCGGGCGAGC CCGCGGAGGT AGGGGGGGCT  
TACGTAGATT TTCGCCACTG CGCCCGCTCG GGGGCCTCCA TCCCCCGCA

8151 CCGGACCCGC CGGGAGAGGG GGCAGGGGCA CGTCGGCGCC GCGCGCGGGC  
GGCCTGGGCG GCCCTCTCCC CGTCCCCGT GCAGCCGCGG CGCGCGCCCG

8201 AGGAGCTGGT GCTGCGCGCG TAGGTTGCTG GCGAACGCGA CGACGCGGCG  
TCCTCGACCA CGACGCGCGC ATCCAACGAC CGCTTGCCT GCTGCGCCGC

8251 GTTGATCTCC TGAATCTGGC GCCTCTGCGT GAAGACGACG GGCCCGGTGA  
CAACTAGAGG ACTTAGACCG CGGAGACGCA CTTCTGCTGC CCGGGCCACT

8301 GCTTGAACCT GAAAGAGAGT TCGACAGAAT CAATTTGCGT GTCGTTGACG  
CGAACTTGA CTTTCTCTCA AGCTGTCTTA GTTAAAGCCA CAGCAACTGC

8351 GCGGCCTGGC GCAAAATCTC CTGCACGTCT CCTGAGTTGT CTTGATAGGC  
CGCCGGACCG CGTTTTAGAG GACGTGCAGA GGAATCAACA GAACTATCCG

8401 GATCTCGGCC ATGAACTGCT CGATCTCTTC CTCCTGGAGA TCTCCGCGTC  
CTAGAGCCGG TACTTGACGA GCTAGAGAAG GAGGACCTCT AGAGGCGCAG

FIG.9A-10

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8451 CGGCTCGCTC CACGGTGGCG GCGAGGTCGT TGGAAATGCG GGCCATGAGC  
 GCCGAGCGAG GTGCCACCGC CGCTCCAGCA ACCTTTACGC CCGGTA CTCTCG

8501 TGCGAGAAGG CGTTGAGGCC TCCCTCGTTC CAGACGCGGC TG TAGACCA C  
 ACGCTCTTCC GCAACTCCGG AGGGAGCAAG GTCTGCGCCG ACATCTGGTG

8551 GCCCCCTTCG GCATCGCGGG CGCGCATGAC CACCTGCGCG AGATTGAGCT  
 CGGGGGAAGC CGTAGCGCCC GCGCGTACTG GTGGACGCGC TCTAACTCGA

8601 CCACGTGCCG GCGAAGACG GCGTAGTTTC GCAGGCGCTG AAAGAGGTAG  
 GGTGCACGGC CCGCTTCTGC CGCATCAAAG CGTCCGCGAC TTTCTCCATC

8651 TTGAGGGTGG TGGCGGTGTG TTCTGCCACG AAGAAGTACA TAACCCAGCG  
 AACTCCCACC ACCGCCACAC AAGACGGTGC TTCTTCATGT ATTGGGTGCG

8701 TCGCAACGTG GATTCGTTGA TATCCCCCAA GGCCTCAAGG CGCTCCATGG  
 AGCGTTGCAC CTAAGCAACT ATAGGGGGTT CCGGAGTTCC GCGAGGTACC

8751 CCTCGTAGAA GTCCACGGCG AAGTTGAAAA ACTGGGAGTT GCGCGCCGAC  
 GGAGCATCTT CAGGTGCCGC TTCAACTTTT TGACCCTCAA CGCGCGGCTG

8801 ACGGTAACT CCTCCTCCAG AAGACGGATG AGCTCGGCGA CAGTGTCGCG  
 TGCCAATTGA GGAGGAGGTC TTCTGCCTAC TCGAGCCGCT GTCACAGCGC

8851 CACCTCGCGC TCAAAGGCTA CAGGGGCCTC TTCTTCTTCT TCAATCTCCT  
 GTGGAGCGCG AGTTTCCGAT GTCCCCGGAG AAGAAGAAGA AGTTAGAGGA

8901 CTTCCATAAG GGCTCCCTC TCTTCTTCTT CTGGCGGCGG TGGGGGAGGG  
 GAAGGTATTC CCGGAGGGGA AGAAGAAGAA GACCGCCGCC ACCCCTCCC

8951 GGGACACGGC GCGACGACG GCGCACC GGG AGGCGGTCGA CAAAGCGCTC  
 CCCTGTGCCG CCGCTGCTGC CGCGTGGCCC TCCGCCAGCT GTTTCGCGAG

9001 GATCATCTCC CCGCGGCGAC GCGCATGGT CTCGGTGACG GCGCGGCCGT  
 CTAGTAGAGG GCGCGCGCTG CCGCGTACCA GAGCCACTGC CGCGCCGGCA

9051 TCTCGCGGGG GCGCAGTTGG AAGACGCCGC CCGTCATGTC CCGGTTATGG  
 AGAGCGCCCC CGCGTCAACC TTCTGCGGCG GGCAGTACAG GGCCAATACC

9101 GTTGGCGGGG GGCTGCCATG CGGCAGGGAT ACGGCGCTAA CGATGCATCT  
 CAACCGCCCC CCGACGGTAC GCCGTCCCTA TGCCGCGATT GCTACGTAGA

9151 CAACAATTGT TGTGTAGGTA CTCCGCCGCC GAGGGACCTG AGCGAGTCCG  
 GTTGTTAACA ACACATCCAT GAGGCGGCGG CTCCCTGGAC TCGCTCAGGC

9201 CATCGACCGG ATCGGAAAAC CTCTCGAGAA AGGCGTCTAA CCAGTCACAG  
 GTAGCTGGCC TAGCCTTTTG GAGAGCTCTT TCCGCAGATT GGTCA GTGTC

9251 TCGCAAGGTA GGCTGAGCAC CGTGGCGGGC GGCAGCGGGC GCGGGTCGGG  
 AGCGTTCCAT CCGACTCGTG GCACCGCCCC CCGTCGCCCC CCGCCAGCCC

FIG.9A-11

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9301 GTTGTTTCTG GCGGAGGTGC TGCTGATGAT GTAATTAAAG TAGGCGGTCT  
 CAACAAAGAC CGCCTCCACG ACGACTACTA CATTAATTTT ATCCGCCAGA  
 9351 TGAGACGGCG GATGGTCGAC AGAAGCACCA TGTCTTGGG TCCGGCCTGC  
 ACTCTGCCGC CTACCAGCTG TCTTCGTGGT ACAGGAACCC AGGCCGGACG  
 9401 TGAATGCGCA GCGGGTCGGC CATGCCCCAG GCTTCGTTTT GACATCGGCG  
 ACTTACGCGT CCGCCAGCCG GTACGGGGTC CGAAGCAAAA CTGTAGCCGC  
 9451 CAGGTCTTTG TAGTAGTCTT GCATGAGCCT TTCTACCGGC ACTTCTTCTT  
 GTCCAGAAAC ATCATCAGAA CGTACTCGGA AAGATGGCCG TGAAGAAGAA  
 9501 CTCCTTCCTC TTGTCCTGCA TCTCTTGCAT CTATCGCTGC GCGGGCGGCG  
 GAGGAAGGAG AACAGGACGT AGAGAACGTA GATAGCGACG CCGCCGCCGC  
 9551 GAGTTTGGCC GTAGGTGGCG CCTCTTCCT CCCATGCGTG TGACCCCGAA  
 CTCAAACCGG CATCCACCGC GGGAGAAGGA GGGTACGCAC ACTGGGGCTT  
 9601 GCCCCTCATC GGCTGAAGCA GGGCTAGGTC GGCACAAACG CGCTCGGCTA  
 CGGGGAGTAG CCGACTTCGT CCCGATCCAG CCGCTGTTGC GCGAGCCGAT  
 9651 ATATGGCCTG CTGCACCTGC GTGAGGGTAG ACTGGAAGTC ATCCATGTCC  
 TATACCGGAC GACGTGGACG CACTCCCATC TGACCTTCAG TAGGTACAGG  
 9701 ACAAAGCGGT GGTATGCGCC CGTGTTGATG GTGTAAGTGC AGTTGGCCAT  
 TGTTTCGCCA CCATACGCGG GCACAACCTAC CACATTCACG TCAACCGGTA  
 9751 AACGGACCAG TTAACGGTCT GGTGACCCGG CTGCGAGAGC TCGGTGTACC  
 TTGCCTGGTC AATTGCCAGA CCACTGGGCC GACGCTCTCG AGCCACATGG  
 9801 TGAGACGCGA GTAAGCCCTC GAGTCAAATA CGTAGTCGTT GCAAGTCCGC  
 ACTCTGCGCT CATTGCGGAG CTCAGTTTAT GCATCAGCAA CGTTCAGGCG  
 9851 ACCAGGTACT GGTATCCAC CAAAAAGTGC GCGGGCGGCT GGCGGTAGAG  
 TGGTCCATGA CCATAGGGTG GTTTTTACG CCGCCGCCGA CCGCCATCTC  
 9901 GGGCCAGCGT AGGGTGGCCG GGGCTCCGGG GGCAGATCT TCCAACATAA  
 CCCGGTCGCA TCCCACCGGC CCCGAGGCC CCGCTCTAGA AGGTTGTATT  
 9951 GGCGATGATA TCCGTAGATG TACCTGGACA TCCAGGTGAT GCCGGCGGCG  
 CCGCTACTAT AGGCATCTAC ATGGACCTGT AGGTCCACTA CGGCCGCCGC  
 10001 GTGGTGGAGG CGCGCGGAAA GTCGCGGACG CGGTTCCAGA TGTTGCGCAG  
 CACCACCTCC GCGCGCCTTT CAGCGCCTGC GCCAAGGTCT ACAACGCGTC  
 10051 CGGCAAAAAG TGCTCCATGG TCGGGACGCT CTGGCCGGTC AGGCGCGCGC  
 GCCGTTTTTC ACGAGGTACC AGCCCTGCGA GACCGGCCAG TCCGCGCGCG  
 10101 AATCGTTGAC GCTCTAGACC GTGCAAAAGG AGAGCCTGTA AGCGGGCACT  
 TTAGCAACTG CGAGATCTGG CACGTTTTCC TCTCGGACAT TCGCCCGTGA

FIG.9A-12



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10151 CTTCCGTGGT CTGGTGGATA AATTCGCAAG GGTATCATGG CGGACGACCG  
GAAGGCACCA GACCACCTAT TTAAGCGTTC CCATAGTACC GCCTGCTGGC

10201 GGGTTCGAGC CCCGTATCCG GCCGTCCGCC GTGATCCATG CGGTTACCGC  
CCCAAGCTCG GGGCATAGGC CGGCAGGCGG CACTAGGTAC GCCAATGGCG

10251 CCGCGTGTCTG AACCCAGGTG TGCACGTCA GACAACGGGG GAGTGCTCCT  
GGCGCACAGC TTGGGTCCAC ACGCTGCAGT CTGTTGCCCC CTCACGAGGA

10301 TTTGGCTTCC TTCCAGGCGC GGCGGCTGCT GCGCTAGCTT TTTTGGCCAC  
AAACCGAAGG AAGGTCCGCG CCGCCGACGA CCGATCGAA AAAACCGGTG

10351 TGGCCGCGCG CAGCGTAAGC GGTTAGGCTG GAAAGCGAAA GCATTAAGTG  
ACCGGCGCGC GTCGCATTCT CCAATCCGAC CTTTCGCTTT CGTAATTCAC

10401 GCTCGCTCCC TGAGCCGGA GGGTTATTTT CCAAGGGTTG AGTCGCGGGA  
CGAGCGAGGG ACATCGGCCT CCAATAAAA GGTTCCTAAC TCAGCGCCCT

10451 CCCCCGTTTC GAGTCTCGGA CCGGCCGGAC TGCGGCGAAC GGGGGTTTGC  
GGGGGCCAAG CTCAGAGCCT GGCCGGCCTG ACGCCGCTTG CCCCCAAACG

10501 CTCCCCGTCA TGCAAGACCC CGCTTGCAAA TTCCTCCGGA AACAGGGACG  
GAGGGGCAGT ACGTTCTGGG GCGAACGTTT AAGGAGGCCT TTGTCCCTGC

10551 AGCCCCTTTT TTGCTTTTCC CAGATGCATC CGGTGCTGCG GCAGATGCGC  
TCGGGGAAAA AACGAAAAGG GTCTACGTAG GCCACGACGC CGTCTACGCG

10601 CCCCTCCTC AGCAGCGGCA AGAGCAAGAG CAGCGGCAGA CATGCAGGGC  
GGGGGAGGAG TCGTCGCCGT TCTCGTTCTC GTCGCCGTCT GTACGTCCCG

10651 ACCCTCCCCT CCTCCTACCG CGTCAGGAGG GCGACATCC GCGGTTGACG  
TGGGAGGGGA GGAGGATGGC GCAGTCCTCC CCGCTGTAGG CGCCACTGC

10701 CGGCAGCAGA TGGTGATTAC GAACCCCGC GCGCCGGGC CCGGCACTAC  
GCCGTGCTCT ACCACTAATG CTTGGGGGCG CCGCGGCCCG GGCCGTGATG

10751 CTGGACTTGG AGGAGGGCGA GGGCCTGGCG CGGCTAGGAG CGCCCTCTCC  
GACCTGAACC TCCTCCCGCT CCCGGACCGC GCCGATCCTC GCGGGAGAGG

10801 TGAGCGGCAC CCAAGGGTGC AGCTGAAGCG TGATACGCGT GAGGCGTACG  
ACTCGCCGTG GGTTCCACG TCGACTTCGC ACTATGCGCA CTCCGCATGC

10851 TGCCGCGGCA GAACCTGTTT CGCGACCGCG AGGGAGAGGA GCCCGAGGAG  
ACGGCGCCGT CTTGGACAAA GCGCTGGCGC TCCCTCTCTC CGGGCTCTC

10901 ATGCGGGATC GAAAGTTCCA CGCAGGGCGC GAGCTGCGGC ATGGCCTGAA  
TACGCCCTAG CTTTCAAGGT GCGTCCCGC CTCGACGCCG TACCGGACTT

10951 TCGCGAGCGG TTGCTGCGCG AGGAGGACTT TGAGCCCGAC GCGCGAACCG  
AGCGCTCGCC AACGACGCGC TCCTCCTGAA ACTCGGGCTG CCGCTTGGC

FIG.9A-13

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11001 GGATTAGTCC CGCGCGCGCA CACGTGGCGG CCGCCGACCT GGTAACCGCA  
 CCTAATCAGG GCGCGCGCGT GTGCACCGCC GGCGGCTGGA CCATTGGCGT

11051 TACGAGCAGA CGGTGAACCA GGAGATTAAC TTTCAAAAAA GCTTTAACAA  
 ATGCTCGTCT GCCACTTGGT CCTCTAATTG AAAGTTTTTT CGAAATTGTT

11101 CCACGTGCGT ACGCTTGTGG CGCGCGAGGA GGTGGCTATA GGAAGTATGC  
 GGTGCACGCA TCGGAACACC GCGCGCTCCT CCACCGATAT CCTGACTACG

11151 ATCTGTGGGA CTTTGTAAAGC GCGCTGGAGC AAAACCCAAA TAGCAAGCCG  
 TAGACACCCT GAAACATTCT CGCGACCTCG TTTTGGGTTT ATCGTTCCGG

11201 CTCATGGCGC AGCTGTTCTT TATAGTGCAG CACAGCAGGG ACAACGAGGC  
 GAGTACCGCG TCGACAAGGA ATATCACGTC GTGTCGTCCC TGTGCTCCC

11251 ATTCAGGGAT GCGCTGCTAA ACATAGTAGA GCGCGAGGGC CGCTGGCTGC  
 TAAGTCCCTA CGCGACGATT TGTATCATCT CGGGCTCCCG GCGACCGACG

11301 TCGATTTGAT AAACATCCTG CAGAGCATAG TGGTGCAGGA GCGCAGCTTG  
 AGCTAAACTA TTTGTAGGAC GTCTCGTATC ACCACGTCCT CGCGTCGAAC

11351 AGCCTGGCTG ACAAGGTGGC CGCCATCAAC TATTCCATGC TTAGCCTGGG  
 TCGGACCGAC TGTTCCACCG GCGGTAGTTG ATAAGGTACG AATCGGACCC

11401 CAAGTTTTAC GCCCGCAAGA TATACCATAC CCCTTACGTT CCCATAGACA  
 GTTCAAAATG CGGGCGTTCT ATATGGTATG GGAATGCAA GGGTATCTGT

11451 AGGAGGTAAA GATCGAGGGG TTCTACATGC GCATGGCGCT GAAGGTGCTT  
 TCCTCCATTT CTAGCTCCCC AAGATGTACG CGTACCGCGA CTTCCACGAA

11501 ACCTTGAGCG ACGACCTGGG CGTTTATCGC AACGAGCGCA TCCACAAGGC  
 TGGAACTCGC TGCTGGACCC GCAAATAGCG TTGCTCGCGT AGGTGTTCCG

11551 CGTGAGCGTG AGCCGGCGGC GCGAGCTCAG CGACCGCGAG CTGATGCACA  
 GCACTCGCAC TCGGCCGCCG CGCTCGAGTC GCTGGCGCTC GACTACGTGT

11601 GCCTGCAAAG GGCCCTGGCT GGCACGGGCA GCGGCGATAG AGAGGCCGAG  
 CGGACGTTTC CCGGGACCGA CCGTGCCCGT CGCCGCTATC TCTCCGGCTC

11651 TCCTACTTTG ACGCGGGCGC TGACCTGCGC TGGGCCCAA GCGGACGCGC  
 AGGATGAAAC TCGGCCCCGCG ACTGGACGCG ACCCGGGGTT CGGCTGCGCG

11701 CCTGGAGGCA GCTGGGGCCG GACCTGGGCT GGCGGTGGCA CCCGCGCGCG  
 GGACCTCCGT CGACCCCGGC CTGGACCCGA CCGCCACCGT GGGCGCGCGC

11751 CTGGCAACGT CGGCGGCGTG GAGGAATATG ACGAGGACGA TGAGTACGAG  
 GACCGTTGCA GCCGCCGCAC CTCCTTATAC TGCTCCTGCT ACTCATGCTC

11801 CCAGAGGACG GCGAGTACTA AGCGGTGATG TTTCTGATCA GATGATGCAA  
 GGTCTCCTGC CGCTCATGAT TCGCCACTAC AAAGACTAGT CTAACGCTT

FIG.9A-14

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11851 GACGCAACGG ACCCGGCGGT GCGGGCGGCG CTGCAGAGCC AGCCGTCCGG  
 CTGCGTTGCC TGGGCCGCCA CGCCGCGCG GACGTCTCGG TCGGCAGGCC  
 11901 CCTTAACTCC ACGGACGACT GGC GCCAGGT CATGGACCGC ATCATGTGCG  
 GGAATTGAGG TGCCTGCTGA CCGCGGTCCA GTACCTGGCG TAGTACAGCG  
 11951 TGA CTGCGCG CAATCCTGAC GCGTTCCGGC AGCAGCCGCA GGCCAACCGG  
 ACTGACGCGC GTTAGGACTG CGCAAGGCCG TCGTCGGCGT CCGGTTGGCC  
 12001 CTCTCCGCAA TTCTGGAAGC GGTGGTCCCG GCGCGCGCAA ACCCCACGCA  
 GAGAGGCGTT AAGACCTTCG CCACCAGGGC CGCGCGCGTT TGGGGTGCCT  
 12051 CGAGAAGGTG CTGGCGATCG TAAACGCGCT GGCCGAAAAC AGGGCCATCC  
 GCTCTTCCAC GACCGCTAGC ATTTGCGCGA CCGGCTTTTG TCCCGGTAGG  
 12101 GGCCCGACGA GGCCGGCCTG GTCTACGACG CGCTGCTTCA GCGCGTGGCT  
 CCGGGCTGCT CCGGCCGGAC CAGATGCTGC GCGACGAAGT CGCGCACCGA  
 12151 CGTTACAACA GCGGCAACGT GCAGACCAAC CTGGACCGGC TGGTGGGGGA  
 GCAATGTTGT CGCCGTTGCA CGTCTGGTTG GACCTGGCCG ACCACCCCT  
 12201 TGTGCGCGAG GCCGTGGCGC AGCGTGAGCG CGCGCAGCAG CAGGGCAACC  
 ACACGCGCTC CGGCACCGCG TCGCACTCGC GCGCGTCGTC GTCCCGTTGG  
 12251 TGGGCTCCAT GGTTGCACTA AACGCCTTCC TGAGTACACA GCCCGCCAAC  
 ACCCGAGGTA CCAACGTGAT TTGCGGAAGG ACTCATGTGT CGGGCGGTTG  
 12301 GTGCCGCGGG GACAGGAGGA CTACACCAAC TTTGTGAGCG CACTGCGGCT  
 CACGGCGCCC CTGTCTCTCT GATGTGGTTG AAACACTCGC GTGACGCCGA  
 12351 AATGGTGACT GAGACACCGC AAAGTGAGGT GTACCAGTCT GGGCCAGACT  
 TTACCACTGA CTCTGTGGCG TTTCACTCCA CATGGTCAGA CCCGGTCTGA  
 12401 ATTTTTTCCA GACCAGTAGA CAAGGCCTGC AGACCGTAAA CCTGAGCCAG  
 TAAAAAAGGT CTGGTCATCT GTTCCGGACG TCTGGCATT TGGACTCGGTC  
 12451 GCTTTTCAAAA ACTTGCAGGG GCTGTGGGGG GTGCGGGCTC CCACAGGCGA  
 CGAAAGTTTT TGAACGTCCC CGACACCCCC CACGCCCAG GGTGTCCGCT  
 12501 CCGCGCGACC GTGTCTAGCT TGCTGACGCC CAACTCGCGC CTGTTGCTGC  
 GGC GCGCTGG CACAGATCGA ACGACTGCGG GTTGAGCGCG GACAACGACG  
 12551 TGCTAATAGC GCCCTTACG GACAGTGGCA GCGTGTCCCG GGACACATAC  
 ACGATTATCG CGGGAAGTGC CTGTCACCGT CGCACAGGGC CCTGTGTATG  
 12601 CTAGGTCACT TGCTGACACT GTACCGCGAG GCCATAGGTC AGGCGCATGT  
 GATCCAGTGA ACGACTGTGA CATGGCGCTC CCGTATCCAG TCCGCGTACA  
 12651 GGACGAGCAT ACTTTCCAGG AGATTACAAG TGTGAGCCGC GCGCTGGGGC  
 CCTGCTCGTA TGAAAGGTCC TCTAATGTT ACAGTCGGCG CGCGACCCCC

FIG.9A-15

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12701 AGGAGGACAC GGGCAGCCTG GAGGCAACCC TAAACTACCT GCTGACCAAC  
TCCTCCTGTG CCCGTCGGAC CTCGGTTGGG ATTTGATGGA CGACTGGTTG

12751 CGGCGGCAGA AGATCCCCTC GTTGCACAGT TTAAACAGCG AGGAGGAGCG  
GCCGCCGTCT TCTAGGGGAG CAACGTGTCA AATTTGTCGC TCCTCCTCGC

12801 CATTTTGCGC TACGTGCAGC AGAGCGTGAG CCTTAACCTG ATGCGCGACG  
GTAAAACGCG ATGCACGTGC TCTCGCACTC GGAATTGGAC TACGCGCTGC

12851 GGGTAACGCC CAGCGTGGCG CTGGACATGA CCGCGCGCAA CATGGAACCG  
CCCATTGCGG GTCGCACCGC GACCTGTACT GCGCGCGTT GTACCTTGGC

12901 GGCATGTATG CCTCAAACCG GCCGTTTATC AACCGCCTAA TGGACTACTT  
CCGTACATAC GGAGTTTGGC CGGCAAATAG TTGGCGGATT ACCTGATGAA

12951 GCATCGCGCG GCCGCCGTGA ACCCCGAGTA TTTCACCAAT GCCATCTTGA  
CGTAGCGCGC CGGCGGCACT TGGGGCTCAT AAAGTGGTTA CGGTAGAACT

13001 ACCCGCACTG GCTACCGCCC CCTGGTTTCT ACACCGGGGG ATTCGAGGTG  
TGGGCGTGAC CGATGGCGGG GGACCAAAGA TGTGGCCCCC TAAGCTCCAC

13051 CCCGAGGGTA ACGATGGATT CCTCTGGGAC GACATAGACG ACAGCGTGTT  
GGGCTCCCAT TGCTACCTAA GGAGACCCTG CTGTATCTGC TGTCGCACAA

13101 TTCCCCGCAA CCGCAGACCC TGCTAGAGTT GCAACAGCGC GAGCAGGCAG  
AAGGGGCGTT GCGTCTGGG ACGATCTCAA CGTTGTCGCG CTCGTCCGTC

13151 AGGCGGCGCT GCGAAAGGAA AGCTTCCGCA GGCCAAGCAG CTTGTCCGAT  
TCCGCCGCGA CGCTTTCCTT TCGAAGGCGT CCGGTTCTGC GAACAGGCTA

13201 CTAGGCGCTG CGGCCCGCG GTCAGATGCT AGTAGCCCAT TTCCAAGCTT  
GATCCGCGAC GCCGGGGCGC CAGTCTACGA TCATCGGGTA AAGGTTCTGA

13251 GATAGGGTCT CTTACCAGCA CTCGCACCAC CCGCCCGCGC CTGCTGGGCG  
CTATCCCAGA GAATGGTCGT GAGCGTGGTG GCGGGGCGCG GACGACCCGC

13301 AGGAGGAGTA CCTAAACAAC TCGCTGCTGC AGCCGCAGCG CGAAAAAAC  
TCCTCCTCAT GGATTTGTTG AGCGACGACG TCGGCGTCGC GCTTTTTTTG

13351 CTGCCTCCGG CATTTCCCAA CAACGGGATA GAGAGCCTAG TGGACAAGAT  
GACGGAGGCC GTAAAGGGTT GTTGCCCTAT CTCTCGGATC ACCTGTTCTA

13401 GAGTAGATGG AAGACGTACG CGCAGGAGCA CAGGGACGTG CCAGGCCCGC  
CTCATCTACC TTCTGCATGC GCGTCTCGT GTCCCTGCAC GGTCGGGGCG

13451 GCCCGCCAC CCGTCGTCAA AGGCACGACC GTCAGCGGGG TCTGGTGTGG  
CGGGCGGGTG GGCAGCAGTT TCCGTGCTGG CAGTCGCCCC AGACCACACC

13501 GAGGACGATG ACTCGGCAGA CGACAGCAGC GTCCTGGATT TGGGAGGGAG  
CTCCTGCTAC TGAGCCGTCT GCTGTCGTCG CAGGACCTAA ACCCTCCCTC

FIG.9A-16

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13551 TGGCAACCCG TTTGCGCACC TTCGCCCCAG GCTGGGGAGA ATGTTTTTAA  
 ACCGTTGGGC AAACGCGTGG AAGCGGGGTC CGACCCCTCT TACAAAATTT  
 13601 AAAAAAAAAA GCATGATGCA AAATAAAAAA CTCACCAAGG CCATGGCACC  
 TTTTTTTTTT CGTACTACGT TTTATTTTTT GAGTGGTTCC GGTACCGTGG  
 13651 GAGCGTTGGT TTTCTTGTAT TCCCCTTAGT ATGCGGCGCG CGGCGATGTA  
 CTCGCAACCA AAAGAACATA AGGGGAATCA TACGCCGCGC GCCGCTACAT  
 13701 TGAGGAAGGT CCTCCTCCCT CCTACGAGAG TGTGGTGAGC GCGGCGCCAG  
 ACTCCTTCCA GGAGGAGGGA GGATGCTCTC ACACCACTCG CGCCGCGGTG  
 13751 TGGCGGCGGC GCTGGGTTCT CCCTTCGATG CTCCCCTGGA CCCGCCGTTT  
 ACCGCCGCCG CGACCCAAGA GGAAGCTAC GAGGGGACCT GGGCGGCAAA  
 13801 GTGCCTCCGC GGTACCTGCG GCCTACCGGG GGGAGAAACA GCATCCGTTA  
 CACGGAGGCG CCATGGACGC CGGATGGCCC CCCTCTTTGT CGTAGGCAAT  
 13851 CTCTGAGTTG GCACCCCTAT TCGACACCAC CCGTGTGTAC CTGGTGGACA  
 GAGACTCAAC CGTGGGGATA AGCTGTGGTG GGCACACATG GACCACCTGT  
 13901 ACAAGTCAAC GGATGTGGCA TCCCTGAACT ACCAGAACGA CCACAGCAAC  
 TGTTCAAGTTG CCTACACCGT AGGGACTTGA TGGTCTTGCT GGTGTCGTTG  
 13951 TTTCTGACCA CGGTCATTCA AAACAATGAC TACAGCCCGG GGGAGGCAAG  
 AAAGACTGGT GCCAGTAAGT TTTGTTACTG ATGTCGGGCC CCCTCCGTTT  
 14001 CACACAGACC ATCAATCTTG ACGACCGGTC GCACTGGGGC GGCACCTGA  
 GTGTGTCTGG TAGTTAGAAC TGCTGGCCAG CGTGACCCCG CCGCTGGACT  
 14051 AAACCATCCT GCATACCAAC ATGCCAAATG TGAACGAGTT CATGTTTACC  
 TTTGGTAGGA CGTATGGTTG TACGGTTTAC ACTTGCTCAA GTACAAATGG  
 14101 AATAAGTTTA AGGCGCGGGT GATGGTGTGCG CGCTTGCCTA CTAAGGACAA  
 TTATTCAAAT TCCGCGCCCA CTACCACAGC GCGAACGGAT GATTCTGTGTT  
 14151 TCAGGTGGAG CTGAAATACG AGTGGGTGGA GTTCACGCTG CCCGAGGGCA  
 AGTCCACCTC GACTTTATGC TCACCCACCT CAAGTGCGAC GGGTCCCCGT  
 14201 ACTACTCCGA GACCATGACC ATAGACCTTA TGAACAACGC GATCGTGGAG  
 TGATGAGGCT CTGGTACTGG TATCTGGAAT ACTTGTTGCG CTAGCACCTC  
 14251 CACTACTTGA AAGTGGGCAG ACAGAACGGG GTTCTGGAAA GCGACATCGG  
 GTGATGAACT TTCACCCGTC TGTCTTGCCC CAAGACCTTT CGCTGTAGCC  
 14301 GGTAAAGTTT GACACCCGCA ACTTCAGACT GGGGTTTGAC CCCGTCACTG  
 CCATTTCAAA CTGTGGGCGT TGAAGTCTGA CCCCAACTG GGGCAGTGAC  
 14351 GTCTTGTCAT GCCTGGGGTA TATACAAACG AAGCCTTCCA TCCAGACATC  
 CAGAACAGTA CGGACCCCAT ATATGTTTGC TTCGGAAGGT AGGTCTGTAG

FIG.9A-17

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14401 ATTTTGCTGC CAGGATGCGG GGTGGACTTC ACCCACAGCC GCCTGAGCAA  
 TAAAACGACG GTCCTACGCC CCACCTGAAG TGGGTGTCGG CGGACTCGTT  
 14451 CTTGTTGGGC ATCCGCAAGC GGCAACCCTT CCAGGAGGGC TTTAGGATCA  
 GAACAACCCG TAGGCGTTCC CCGTTGGGAA GGTCTCCCG AAATCCTAGT  
 14501 CCTACGATGA TCTGGAGGGT GGTAACATTC CCGCACTGTT GGATGTGGAC  
 GGATGCTACT AGACCTCCCA CCATTGTAAG GCGTGACAA CCTACACCTG  
 14551 GCCTACCAGG CGAGCTTGAA AGATGACACC GAACAGGGCG GGGGTGGCGC  
 CGGATGGTCC GCTCGAATT TCTACTGTGG CTTGTCCCGC CCCACCGCG  
 14601 AGGCGGCAGC AACAGCAGTG GCAGCGGCGC GGAAGAGAAC TCCAACGCGG  
 TCCGCCGTCG TTGTCGTCAC CGTCGCCGCG CTTCTCTTG AGGTTGCGCC  
 14651 CAGCCGCGGC AATGCAGCCG GTGGAGGACA TGAACGATCA TGCCATTCGC  
 GTCGGCGCCG TTACGTCGGC CACCTCCTGT ACTTGCTAGT ACGGTAAGCG  
 14701 GGCACACCT TTGCCACACG GGCTGAGGAG AAGCGCGCTG AGGCCGAAGC  
 CCGCTGTGGA AACGGTGTGC CCGACTCCTC TTCGCGCGAC TCCGGCTTCG  
 14751 AGCGGCCGAA GCTGCCGCCC CCGCTGCGCA ACCCGAGGTC GAGAAGCCTC  
 TCGCCGGCTT CGACGGCGGG GCGACGCGT TGGGCTCCAG CTCTTCGGAG  
 14801 AGAAGAAACC GGTGATCAAA CCCCTGACAG AGGACAGCAA GAAACGCAGT  
 TCTTCTTTGG CCACTAGTTT GGGGACTGTC TCCTGTCGTT CTTTGCGTCA  
 14851 TACAACCTAA TAAGCAATGA CAGCACCTTC ACCCAGTACC GCAGCTGGTA  
 ATGTTGGATT ATTCGTTACT GTCGTGGAAG TGGGTCATGG CGTCGACCAT  
 14901 CCTTGCATAC AACTACGGCG ACCCTCAGAC CGGAATCCGC TCATGGACCC  
 GGAACGTATG TTGATGCCGC TGGGAGTCTG GCCTTAGGCG AGTACCTGGG  
 14951 TGCTTTGCAC TCCTGACGTA ACCTGCGGCT CGGAGCAGGT CTACTGGTCG  
 ACGAAACGTG AGGACTGCAT TGGACGCCGA GCCTCGTCCA GATGACCAGC  
 15001 TTGCCAGACA TGATGCAAGA CCCCCTGACC TTCCGCTCCA CGCGCCAGAT  
 AACGGTCTGT ACTACGTTCT GGGGCACTGG AAGGCGAGGT GCGCGGTCTA  
 15051 CAGCAACTTT CCGGTGGTGG GCGCCGAGCT GTTGCCCGTG CACTCCAAGA  
 GTCGTTGAAA GGCCACCACC CGCGGCTCGA CAACGGGCAC GTGAGGTTCT  
 15101 GCTTCTACAA CGACCAGGCC GTCTACTCCC AACTCATCCG CCAGTTTACC  
 CGAAGATGTT GCTGGTCCGG CAGATGAGGG TTGAGTAGGC GGTCAAATGG  
 15151 TCTCTGACCC ACGTGTTCAA TCGCTTTCCC GAGAACCAGA TTTTGGCGCG  
 AGAGACTGGG TGCACAAGTT AGCGAAAGGG CTCTTGGTCT AAAACCGCGC  
 15201 CCCGCCAGCC CCCACCATCA CCACCGTCAG TGAAAACGTT CCTGCTCTCA  
 GGGCGGTCGG GGTGGTAGT GGTGGCAGTC ACTTTTGCAA GGACGAGAGT

FIG.9A-18

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15251 CAGATCACGG GACGCTACCG CTGCGCAACA GCATCGGAGG AGTCCAGCGA  
GTCTAGTGCC CTGCGATGGC GACGCGTTGT CGTAGCCTCC TCAGGTCGCT

15301 GTGACCATT A CTGACGCCAG ACGCCGCACC TGCCCCTACG TTTACAAGGC  
CACTGGTAAT GACTGCGGTC TGCGGCGTGG ACGGGGATGC AAATGTTCCG

15351 CCTGGGCATA GTCTCGCCGC GCGTCCTATC GAGCCGCACT TTTTGAGCAA  
GGACCCGTAT CAGAGCGGCG CGCAGGATAG CTCGGCGTGA AAAACTCGTT

15401 GCATGTCCAT CCTTATATCG CCCAGCAATA ACACAGGCTG GGGCCTGCGC  
CGTACAGGTA GGAATATAGC GGGTCGTTAT TGTGTCCGAC CCCGGACGCG

15451 TTCCCAAGCA AGATGTTTGG CGGGGCCAAG AAGCGCTCCG ACCAACACCC  
AAGGGTTCGT TCTACAAACC GCCCCGGTTC TTCGCGAGGC TGGTTGTGGG

15501 AGTGCGCGTG CGCGGGCACT ACCGCGCGCC CTGGGGCGCG CACAAACGCG  
TCACGCGCAC GCGCCCGTGA TGGCGCGCGG GACCCCGCGC GTGTTTGC

15551 GCCGCACTGG GCGCACCACC GTCGATGACG CCATCGACGC GGTGGTGGAG  
CGGCGTGACC CGCGTGGTGG CAGCTACTGC GGTAGCTGCG CCACCACCTC

15601 GAGGCGCGCA ACTACACGCC CACGCCGCCA CCAGTGTCCA CAGTGGACGC  
CTCCGCGCGT TGATGTGCGG GTGCGGCGGT GGTACAGGT GTCACCTGCG

15651 GGCCATTAG ACCGTGGTGC GCGGAGCCCG GCGCTATGCT AAAATGAAGA  
CCGGTAAGTC TGGCACCACG CGCCTCGGGC CGCGATACGA TTTTACTTCT

15701 GACGGCGGAG GCGCGTAGCA CGTCGCCACC GCCGCCGACC CGGCACTGCC  
CTGCCGCCTC CGCGCATCGT GCAGCGGTGG CGGCGGCTGG GCCGTGACGG

15751 GCCCAACGCG CGGCGGCGGC CCTGCTTAAC CGCGCACGTC GCACCGGCCG  
CGGGTTGCGC GCCGCCGCCG GGACGAATTG GCGCGTGCGC CGTGGCCGGC

15801 ACGGGCGGCC ATGCGGGCCG CTCGAAGGCT GGCCGCGGGT ATTGTCACTG  
TGCCCGCCGG TACGCCCGGC GAGCTTCCGA CCGCGGCCCA TAACAGTGAC

15851 TGCCCCCAG GTCCAGGCGA CGAGCGGCCG CCGCAGCAGC CGCGGCCATT  
ACGGGGGGTC CAGGTCCGCT GCTCGCCGGC GCGTCGTCG GCGCCGGTAA

15901 AGTGCTATGA CTCAGGGTCG CAGGGGCAAC GTGTATTGGG TGCGCGACTC  
TCACGATACT GAGTCCAGC GTCCCCGTTG CACATAACCC ACGCGCTGAG

15951 GGTTAGCGGC CTGCGCGTGC CCGTGCGCAC CCGCCCCCG CGCAACTAGA  
CCAATCGCCG GACGCGCACG GGCACGCGTG GGCGGGGGGC GCGTTGATCT

16001 TTGCAAGAAA AAATACTTA GACTCGTACT GTTGTATGTA TCCAGCGGCG  
AACGTTCTTT TTTGATGAAT CTGAGCATGA CAACATACAT AGGTCGCCGC

16051 GCGGCGCGCA ACGAAGCTAT GTCCAAGCGC AAAATCAAAG AAGAGATGCT  
CGCCGCGCGT TGCTTCGATA CAGGTTGCGG TTTTAGTTTC TTCTCTACGA

FIG.9A-19

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16101 CCAGGTCATC GCGCCGGAGA TCTATGGCCC CCCGAAGAAG GAAGAGCAGG  
GGTCCAGTAG CGCGGCCTCT AGATACCGGG GGGCTTCTTC CTTCTCGTCC

16151 ATTACAAGCC CCGAAAGCTA AAGCGGGTCA AAAAGAAAAA GAAAGATGAT  
TAATGTTCCG GGCTTTCGAT TTCGCCAGT TTTTCTTTTT CTTTCTACTA

16201 GATGATGAAC TTGACGACGA GGTGGAAGT CTGCACGCTA CCGCGCCCAG  
CTACTACTTG AACTGCTGCT CCACCTTGAC GACGTGCGAT GGCGCGGGTC

16251 GCGACGGGTA CAGTGGAAAG GTCGACGCGT AAAACGTGTT TTGCGACCCG  
CGCTGCCCAT GTCACCTTTC CAGCTGCGCA TTTTGCACAA AACGCTGGGC

16301 GCACCACCGT AGTCTTTACG CCCGGTGAGC GCTCCACCCG CACCTACAAG  
CGTGGTGGCA TCAGAAATGC GGGCCACTCG CGAGGTGGGC GTGGATGTTC

16351 CGCGTGTATG ATGAGGTGTA CGGCGACGAG GACCTGCTTG AGCAGGCCAA  
GCGCACATAC TACTCCACAT GCCGCTGCTC CTGGACGAAC TCGTCCGGTT

16401 CGAGCGCCTC GGGGAGTTTG CCTACGGAAA GCGGCATAAG GACATGCTGG  
GCTCGCGGAG CCCCTCAAAC GGATGCCTTT CGCCGTATTC CTGTACGACC

16451 CGTTGCCGCT GGACGAGGGC AACCACAACAC CTAGCCTAAA GCCCGTAACA  
GCAACGGCGA CCTGCTCCCG TTGGGTTGTG GATCGGATTT CGGGCATTGT

16501 CTGCAGCAGG TGCTGCCCGC GCTTGCACCG TCCGAAGAAA AGCGCGGCCT  
GACGTGCTCC ACGACGGGCG CGAACGTGGC AGGCTTCTTT TCGCGCCGGA

16551 AAAGCGCGAG TCTGGTGACT TGGCACCCAC CGTGCAGCTG ATGGTACCCA  
TTTCGCGCTC AGACCACTGA ACCGTGGGTG GCACGTGAC TACCATGGGT

16601 AGCGCCAGCG ACTGGAAGAT GTCTTGAAAA AAATGACCGT GGAACCTGGG  
TCGCGGTGCG TGACCTTCTA CAGAACCTTT TTTACTGGCA CCTTGACCC

16651 CTGGAGCCCG AGGTCCGCGT GCGGCCAATC AAGCAGGTGG CGCCGGGACT  
GACCTCGGGC TCCAGGCGCA CGCCGGTTAG TTCGTCCACC GCGGCCCTGA

16701 GGGCGTGCAG ACCGTGGACG TTCAGATACC CACTACCAGT AGCACCAGTA  
CCCGCACGTC TGGCACCTGC AAGTCTATGG GTGATGGTCA TCGTGGTCAT

16751 TTGCCACCGC CACAGAGGGC ATGGAGACAC AAACGTCCCC GGTTGCCTCA  
AACGGTGGCG GTGTCTCCCG TACCTCTGTG TTTGCAGGGG CCAACGGAGT

16801 GCGGTGGCGG ATGCCGCGGT GCAGGCGGTC GCTGCGGCCG CGTCCAAGAC  
CGCCACCGCC TACGGCGCCA CGTCCGCCAG CGACGCCGGC GCAGGTTCTG

16851 CTCTACGGAG GTGCAAACGG ACCCGTGGAT GTTTCGCGTT TCAGCCCCC  
GAGATGCCTC CACGTTTGCC TGGGCACCTA CAAAGCGCAA AGTCGGGGGG

16901 GGCGCCCCGCG CCGTTCGAGG AAGTACGGCG CCGCCAGCGC GCTACTGCCC  
CCGCGGGGCG GGCAAGCTCC TTCATGCCGC GCGGTCGCG CGATGACGGG

FIG.9A-20



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16951 GAATATGCCC TACATCCTTC CATTGCGCCT ACCCCCGGCT ATCGTGGCTA  
CTTATACGGG ATGTAGGAAG GTAACGCGGA TGGGGGCCGA TAGCACCGAT

17001 CACCTACCGC CCCAGAAGAC GAGCAACTAC CCGACGCCGA ACCACCACTG  
GTGGATGGCG GGGTCTTCTG CTCGTTGATG GGCTGCGGCT TGGTGGTGAC

17051 GAACCCGCCG CCGCCGTCGC CGTCGCCAGC CCGTGCTGGC CCCGATTTCC  
CTTGGGCGGC GGC GGCGCAGCG GCAGCGGTCTG GGCACGACCG GGGCTAAAGG

17101 GTGCGCAGGG TGGCTCGCGA AGGAGGCAGG ACCCTGGTGC TGCCAACAGC  
CACGCGTCCC ACCGAGCGCT TCCTCCGTCC TGGGACCACG ACGGTTGTCTG

17151 GCGCTACCAC CCCAGCATCG TTTAAAAGCC GGTCTTTGTG GTTCTTGCGA  
CGCGATGGTG GGGTCGTAGC AAATTTTCGG CCAGAAACAC CAAGAACGTC

17201 ATATGGCCCT CACCTGCCGC CTCCGTTTCC CGGTGCCGGG ATTCCGAGGA  
TATACCGGGA GTGGACGGCG GAGGCAAAGG GCCACGGCCC TAAGGCTCCT

17251 AGAATGCACC GTAGGAGGGG CATGGCCGGC CACGGCCTGA CGGGCGGCAT  
TCTTACGTGG CATCCTCCCC GTACCGGCCG GTGCCGGACT GCCCGCCGTA

17301 GCGTCGTGCG CACCACCGGC GCGGCGCGCG GTCGCACCGT CGCATGCGCG  
CGCAGCACGC GTGGTGGCCG CCGCCGCGCG CAGCGTGGCA GCGTACGCGC

17351 GCGGTATCCT GCCCTCCTT ATTCCACTGA TCGCCGCGGC GATTGGCGCC  
CGCCATAGGA CGGGGAGGAA TAAGGTGACT AGCGGCGCCG CTAACCGCGG

17401 GTGCCCCGAA TTGCATCCGT GGCCTTGCGA GCGCAGAGAC ACTGATTAAA  
CACGGGCCCT AACGTAGGCA CCGGAACGTC CGCGTCTCTG TGAATAATTT

17451 AACAAAGTTGC ATGTGGAAAA ATCAAAATAA AAAGTCTGGA CTCTCACGCT  
TTGTTCAACG TACACCTTTT TAGTTTTATT TTTCAGACCT GAGAGTGCGA

17501 CGCTTGGTCC TGTAACATTT TTGTAGAATG GAAGACATCA ACTTTGCGTC  
GCGAACCAGG ACATTGATAA AACATCTTAC CTTCTGTAGT TGAAACGCAG

17551 TCTGGCCCCG CGACACGGCT CGCGCCCGTT CATGGGAAAC TGGCAAGATA  
AGACCGGGGC GCTGTGCCGA GCGCGGGCAA GTACCCTTTG ACCGTTCTAT

17601 TCGGCACCAG CAATATGAGC GGTGGCGCCT TCAGCTGGGG CTCGCTGTGG  
AGCCGTGGTC GTTATACTCG CCACCGCGGA AGTCGACCCC GAGCGACACC

17651 AGCGGCATTA AAAATTTTCGG TTCCACCGTT AAGAACTATG GCAGCAAGGC  
TCGCCGTAAT TTTTAAAGCC AAGGTGGCAA TTCTTGATAC CGTCGTTCCG

17701 CTGGAACAGC AGCACAGGCC AGATGCTGAG GGATAAGTTG AAAGAGCAAA  
GACCTTGTCG TCGTGTCCGG TCTACGACTC CCTATTCAAC TTTCTCGTTT

17751 ATTTCCAACA AAAGGTGGTA GATGGCCTGG CCTCTGGCAT TAGCGGGGTG  
TAAAGGTTGT TTTCCACCAT CTACCGGACC GGAGACCGTA ATCGCCCCAC

FIG.9A-21

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17801 GTGGACCTGG CCAACCAGGC AGTGCAAAAT AAGATTAACA GTAAGCTTGA  
CACCTGGACC GGTGGTCCG TCACGTTTTA TTCTAATTGT CATTGGAAC

17851 TCCCCGCCCT CCCGTAGAGG AGCCTCCACC GGCCGTGGAG ACAGTGTCTC  
AGGGGCGGGA GGGCATCTCC TCGGAGGTGG CCGGCACCTC TGTCACAGAG

17901 CAGAGGGGCG TGGCGAAAAG CGTCCGCGCC CCGACAGGGA AGAACTCTG  
GTCTCCCCGC ACCGCTTTTC GCAGGCGCGG GGCTGTCCCT TCTTTGAGAC

17951 GTGACGCAAA TAGACGAGCC TCCCTCGTAC GAGGAGGCAC TAAAGCAAGG  
CACTGCGTTT ATCTGCTCGG AGGGAGCATG CTCCTCCGTG ATTCGTTCC

18001 CCTGCCCACC ACCCGTCCCA TCGCGCCCAT GGCTACCGGA GTGCTGGGCC  
GGACGGGTGG TGGCAGGGT AGCGCGGGTA CCGATGGCCT CACGACCCGG

18051 AGCACACACC CGTAACGCTG GACCTGCCTC CCCCCGCCGA CACCCAGCAG  
TCGTGTGTGG GCATTGCGAC CTGGACGGAG GGGGGCGGCT GTGGGTGCTC

18101 AAACCTGTGC TGCCAGGCC GACCGCCGTT GTTGTAAACC GTCCTAGCCG  
TTTGACACG ACGGTCCGGG CTGGCGGCAA CAACATTGGG CAGGATCGGC

18151 CGCGTCCCTG CGCCGCGCCG CCAGCGGTCC GCGATCGTTG CGGCCCGTAG  
GCGCAGGGAC GCGGCGCGGC GGTCGCCAGG CGCTAGCAAC GCCGGGCATC

18201 CCAGTGCGAA CTGGCAAAGC AACTGAACA GCATCGTGGG TCTGGGGGTG  
GGTCACCGTT GACCGTTTCG TGTGACTTGT CGTAGCACC AGACCCCAAC

18251 CAATCCCTGA AGCGCCGACG ATGCTTCTGA TAGCTAACGT GTCGTATGTG  
GTTAGGGACT TCGCGGCTGC TACGAAGACT ATCGATTGCA CAGCATACAC

18301 TGTCATGTAT GCGTCCATGT CGCCGCCAGA GGAGCTGCTG AGCCGCCGCG  
ACAGTACATA CGCAGGTACA GCGGCGGTCT CCTCGACGAC TCGGCGGCGC

18351 CGCCCGCTTT CCAAGATGGC TACCCCTTCG ATGATGCCGC AGTGGTCTTA  
GCGGGCGAAA GGTCTACCG ATGGGGAAGC TACTACGGCG TCACCAGAAT

18401 CATGCACATC TCGGGCCAGG ACGCCTCGGA GTACCTGAGC CCCGGGCTGG  
GTACGTGTAG AGCCCGGTCC TGCGGAGCCT CATGGACTCG GGGCCCCGAC

18451 TGCAGTTTGC CCGCGCCACC GAGACGTACT TCAGCCTGAA TAACAAGTTT  
ACGTCAAACG GGC GCGGTGG CTCTGCATGA AGTCGGACTT ATTGTTCAAA

18501 AGAAACCCCA CGGTGGCGCC TACGCACGAC GTGACCACAG ACCGGTCCCA  
TCTTTGGGGT GCCACGCGG ATGCGTGCTG CACTGGTGTC TGCCAGGGT

18551 GCGTTTGACG CTGCGGTTCA TCCCTGTGGA CCGTGAGGAT ACTGCGTACT  
CGCAAACGTC GACGCCAAGT AGGGACACCT GGCACCTCCTA TGACGCATGA

18601 CGTACAAGGC GCGGTTCCACC CTAGCTGTGG GTGATAACCG TGTGCTGGAC  
GCATGTTCCG CGCCAAGTGG GATCGACACC CACTATTGGC ACACGACCTG

FIG.9A-22

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18651 ATGGCTTCCA CGTACTTTGA CATCCGCGGC GTGCTGGACA GGGGCCCTAC  
TACCGAAGGT GCATGAAACT GTAGGCGCCG CACGACCTGT CCCCGGGATG

18701 TTTTAAGCCC TACTCTGGCA CTGCCTACAA CGCCCTGGCT CCCAAGGGTG  
AAAATTCGGG ATGAGACCGT GACGGATGTT GCGGGACCGA GGGTTCCAC

18751 CCCCAAATCC TTGCGAATGG GATGAAGCTG CTA CTGCTCT TGAATAAAC  
GGGGTTTAGG AACGCTTACC CTA CTGCTGAC GATGACGAGA ACTTTATTG

18801 CTAGAAGAAG AGGACGATGA CAACGAAGAC GAAGTAGACG AGCAAGCTGA  
GATCTTCTTC TCCTGCTACT GTTGCTTCTG CTTTCATCTG TCGTTCGACT

18851 GCAGCAAAAA ACTCACGTAT TTGGGCAGGC GCCTTATTCT GGTATAAATA  
CGTCGTTTTT TGAGTGCATA AACCCGTCCG CGGAATAAGA CCATATTTAT

18901 TTACAAAGGA GGGTATTCAA ATAGGTGTCG AAGGTCAAAC ACCTAAATAT  
AATGTTTCCT CCCATAAGTT TATCCACAGC TTCCAGTTTG TGGATTTATA

18951 GCCGATAAAA CATTTCAACC TGAACCTCAA ATAGGAGAAT CTCAGTGGTA  
CGGCTATTTT GTAAAGTTGG ACTTGGAGTT TATCCTCTTA GAGTCACCAT

19001 CGAAACAGAA ATTAATCATG CAGCTGGGAG AGTCCTAAAA AAGACTACCC  
GCTTTGTCTT TAATTAGTAC GTCGACCCTC TCAGGATTTT TTCTGATGGG

19051 CAATGAAACC ATGTTACGGT TCATATGCAA AACCCACAAA TGAAATGGA  
GTTACTTTGG TACAATGCCA AGTATACGTT TTGGGTGTTT ACTTTTACCT

19101 GGGCAAGGCA TTCTTGTAAG GCAACAAAAT GGAAAGCTAG AAAGTCAAGT  
CCCGTTCCGT AAGAACATTT CGTTGTTTTA CCTTTCGATC TTTCAGTTCA

19151 GGAAATGCAA TTTTCTCAA CTA CTGAGGC AGCCGCAGGC AATGGTGATA  
CCTTTACGTT AAAAAGAGTT GATGACTCCG TCGGCGTCCG TTACCACTAT

19201 ACTTGACTCC TAAAGTGGTA TTGTACAGTG AAGATGTAGA TATAGAAACC  
TGAAGTGAAG ATTTACCAT AACATGTCAC TTCTACATCT ATATCTTTGG

19251 CCAGACACTC ATATTTCTTA CATGCCCACT ATTAAGGAAG GTAAGTCAGG  
GGTCTGTGAG TATAAAGAAT GTACGGGTGA TAATTCCTTC CATTGAGTGC

19301 AGAACTAATG GGCCAACAAT CTATGCCCAA CAGGCCTAAT TACATTGCTT  
TCTTGATTAC CCGGTTGTGA GATACGGGTT GTCCGGATTA ATGTAACGAA

19351 TTAGGGACAA TTTTATTGGT CTAATGTATT ACAACAGCAC GGGTAATATG  
AATCCCTGTT AAAATAACCA GATTACATAA TGTTGTCTG CCCATTATAC

19401 GGTGTTCTGG CGGGCCAAGC ATCGCAGTTG AATGCTGTTG TAGATTTGCA  
CCACAAGACC GCGCGGTTG TAGCGTCAAC TTACGACAAC ATCTAAACGT

19451 AGACAGAAAC ACAGAGCTTT CATACCAGCT TTTGCTTGAT TCCATTGGTG  
TCTGTCTTTG TGTCTCGAAA GTATGGTCGA AAACGAACTA AGGTAACCAC

FIG.9A-23

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19501 ATAGAACCAG GTACTTTTCT ATGTGGAATC AGGCTGTTGA CAGCTATGAT  
TATCTTGGTC CATGAAAAGA TACACCTTAG TCCGACAACT GTCGATACTA

19551 CCAGATGTTA GAATTATTGA AAATCATGGA ACTGAAGATG AACTTCCAAA  
GGTCTACAAT CTTAATAACT TTTAGTACCT TGACTTCTAC TTGAAGGTTT

19601 TTA CTGCTTT CCACTGGGAG GTGTGATTAA TACAGAGACT CTTACCAAGG  
AATGACGAAA GGTGACCCTC CACACTAATT ATGTCTCTGA GAATGGTTCC

19651 TAAAACCTAA AACAGGTCAG GAAAATGGAT GGGAAAAAGA TGCTACAGAA  
ATTTTGGATT TTGTCCAGTC CTTTACCTA CCCTTTTCT ACGATGTCTT

19701 TTTTCAGATA AAAATGAAAT AAGAGTTGGA AATAATTTTG CCATGGAAAT  
AAAAGTCTAT TTTTACTTTA TTCTCAACCT TTATTAAAC GGTACCTTTA

19751 CAATCTAAAT GCCAACCTGT GGAGAAATTT CCTGTACTCC AACATAGCGC  
GTTAGATTTA CGGTTGGACA CCTCTTTAAA GGACATGAGG TTGTATCGCG

19801 TGTATTTGCC CGACAAGCTA AAGTACAGTC CTTCCAACGT AAAAATTTCT  
ACATAAACGG GCTGTTTCGAT TTCATGTCAG GAAGGTTGCA TTTTAAAGA

19851 GATAACCCAA ACACCTACGA CTACATGAAC AAGCGAGTGG TGGCTCCCGG  
CTATTGGGTT TGTGGATGCT GATGTACTTG TTCGCTCACC ACCGAGGGCC

19901 GCTAGTGGAC TGCTACATTA ACCTTGGAGC ACGCTGGTCC CTTGACTATA  
CGATCACCTG ACGATGTAAT TGGAACCTCG TGCGACCAGG GAACTGATAT

19951 TGGACAACGT CAACCCATTT AACCACCACC GCAATGCTGG CCTGCGCTAC  
ACCTGTTGCA GTTGGGTAAA TTGGTGGTGG CGTTACGACC GGACGCGATG

20001 CGCTCAATGT TGCTGGGCAA TGGTCGCTAT GTGCCCTTCC ACATCCAGGT  
GCGAGTTACA ACGACCCGTT ACCAGCGATA CACGGGAAGG TGTAGGTCCA

20051 GCCTCAGAAG TTCTTTGCCA TTA AAAACCT CTTCTCCTG CCGGGCTCAT  
CGGAGTCTTC AAGAAACGGT AATTTTGGGA GGAAGAGGAC GGCCCGAGTA

20101 ACACCTACGA GTGGAACCTC AGGAAGGATG TTAACATGGT TCTGCAGAGC  
TGTGGATGCT CACCTTGAAG TCCTTCTAC AATTGTACCA AGACGTCTCG

20151 TCCCTAGGAA ATGACCTAAG GGTGACGGA GCCAGCATT A GTTTGATAG  
AGGGATCCTT TACTGGATTC CCAACTGCCT CGGTCGTAAT TCAAATATC

20201 CATTTGCCTT TACGCCACCT TCTTCCCAT GGCCACAAAC ACCGCCTCCA  
GTAAACGGAA ATGCGGTGGA AGAAGGGGTA CCGGTGTTG TGCGGAGGT

20251 CGCTTGAGGC CATGCTTAGA AACGACACCA ACGACCAGTC CTTTAACGAC  
GCGAACTCCG GTACGAATCT TTGCTGTGGT TGCTGGTCAG GAAATTGCTG

20301 TATCTCTCCG CCGCCAACAT GCTCTACCCT ATACCCGCCA ACGCTACCAA  
ATAGAGAGGC GGCGGTTGTA CGAGATGGGA TATGGGCGGT TGCGATGGTT

FIG.9A-24

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20351 CGTGCCCATATA TCCATCCCCT CCCGCAACTG GGCGGCTTTC CGCGGCTGGG  
GCACGGGTAT AGGTAGGGGA GGGCGTTGAC CCGCCGAAAG GCGCCGACCC

20401 CCTTCACGCG CCTTAAGACT AAGGAAACCC CATCACTGGG CTCGGGCTAC  
GGAAGTGCGC GGAATTCTGA TTCCTTTGGG GTAGTGACCC GAGCCCGATG

20451 GACCCTTATT ACACCTACTC TGGCTCTATA CCCTACCTAG ATGGAACCTT  
CTGGGAATAA TGTGGATGAG ACCGAGATAT GGGATGGATC TACCTTGGAA

20501 TTACCTCAAC CACACCTTTA AGAAGGTGGC CATTACCTTT GACTCTTCTG  
AATGGAGTTG GTGTGGAAAT TCTCCACCG GTAATGGAAA CTGAGAAGAC

20551 TCAGCTGGCC TGGCAATGAC CGCCTGCTTA CCCCCAACGA GTTTGAAATT  
AGTCGACCGG ACCGTTACTG GCGGACGAAT GGGGGTTGCT CAACTTTAA

20601 AAGCGCTCAG TTGACGGGGA GGGTTACAAC GTTGCCAGT GTAACATGAC  
TTCGCGAGTC AACTGCCCCCT CCCAATGTTG CAACGGGTCA CATTGTACTG

20651 CAAAGACTGG TTCCTGGTAC AAATGCTAGC TAACTATAAC ATTGGCTACC  
GTTTCTGACC AAGGACCATG TTTACGATCG ATTGATATTG TAACCGATGG

20701 AGGGCTTCTA TATCCCAGAG AGCTACAAGG ACCGCATGTA CTCCTTCTTT  
TCCCGAAGAT ATAGGGTCTC TCGATGTTCC TGGCGTACAT GAGGAAGAAA

20751 AGAAACTTCC AGCCCATGAG CCGTCAGGTG GTGGATGATA CTAAATACAA  
TCTTTGAAGG TCGGGTACTC GGCAGTCCAC CACCTACTAT GATTTATGTT

20801 GGACTIONACAA CAGGTGGGCA TCCTACACCA ACACAACAAC TCTGGATTG  
CCTGATGGTT GTCCACCCGT AGGATGTGGT TGTGTTGTTG AGACCTAAAC

20851 TTGGCTACCT TGCCCCCACC ATGCGCGAAG GACAGGCCTA CCCTGCTAAC  
AACCATGGA ACGGGGGTGG TACGCGCTTC CTGTCCGGAT GGGACGATTG

20901 TTCCCCTATC CGCTTATAGG CAAGACCGCA GTTGACAGCA TTACCCAGAA  
AAGGGGATAG GCGAATATCC GTTCTGGCGT CAACTGTCGT AATGGGTCTT

20951 AAAGTTTCTT TGCGATCGCA CCCTTTGGCG CATCCCATTC TCCAGTAACT  
TTTCAAAGAA ACGCTAGCGT GGGAAACCGC GTAGGGTAAG AGGTCATTGA

21001 TTATGTCCAT GGGCGCACTC ACAGACCTGG GCCAAAACCT TCTCTACGCC  
AATACAGGTA CCCGCGTGAG TGTCTGGACC CGGTTTTGGA AGAGATGCGG

21051 AACTCCGCCC ACGCGCTAGA CATGACTTTT GAGGTGGATC CCATGGACGA  
TTGAGGCGGG TGCGCGATCT GTACTGAAAA CTCCACCTAG GGTACCTGCT

21101 GCCCACCTT CTTTATGTTT TGTTTGAAGT CTTTGACGTG GTCCGTGTGC  
CGGGTGGGAA GAAATACAAA ACAAACCTCA GAAACTGCAC CAGGCACACG

21151 ACCAGCCGCA CCGCGGCGTC ATCGAAACCG TGTACCTGCG CACGCCCTTC  
TGTGCGGCGT GGCGCCGAG TAGCTTTGGC ACATGGACGC GTGCGGGAAG

FIG.9A-25

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21201 TCGGCCGGCA ACGCCACAAC ATAAAGAAGC AAGCAACATC AACAACAGCT  
AGCCGGCCGT TCGGTGTTG TATTTCTTCG TTCGTTGTAG TTGTTGTCGA

21251 GCCGCCATGG GCTCCAGTGA GCAGGAACTG AAAGCCATTG TCAAAGATCT  
CGGCGGTACC CGAGGTCACT CGTCCTTGAC TTTCGGTAAC AGTTTCTAGA

21301 TGGTTGTGGG CCATATTTTT TGGGCACCTA TGACAAGCGC TTTCCAGGCT  
ACCAACACCC GGTATAAAAA ACCCGTGGAT ACTGTTGCGG AAAGGTCCGA

21351 TTGTTTCTCC ACACAAGCTC GCCTGCGCCA TAGTCAATAC GGCCGGTCGC  
AACAAAGAGG TGTGTTGAG CGGACGCGGT ATCAGTTATG CCGGCCAGCG

21401 GAGACTGGGG GCGTACACTG GATGGCCTTT GCCTGGAACC CGCACTCAAA  
CTCTGACCCC CGCATGTGAC CTACCGGAAA CGGACCTTGG GCGTGAGTTT

21451 AACATGCTAC CTCTTTGAGC CCTTTGGCTT TTCTGACCAG CGACTCAAGC  
TTGTACGATG GAGAACTCG GGAAACCGAA AAGACTGGTC GCTGAGTTCTG

21501 AGGTTTACCA GTTTGAGTAC GAGTCACTCC TGCGCCGTAG CGCCATTGCT  
TCCAAATGGT CAAACTCATG CTCAGTGAGG ACGCGGCATC GCGGTAACGA

21551 TCTTCCCCCG ACCGCTGTAT AACGCTGGAA AAGTCCACCC AAAGCGTACA  
AGAAGGGGGC TGGCGACATA TTGCGACCTT TTCAGGTGGG TTTCGCATGT

21601 GGGGCCCAAC TCGGCCGCTT GTGGACTATT CTGCTGCATG TTTCTCCACG  
CCCCGGGTTG AGCCGGCGGA CACCTGATAA GACGACGTAC AAAGAGGTGC

21651 CCTTTGCCAA CTGGCCCCAA ACTCCCATGG ATCACAACCC CACCATGAAC  
GGAAACGGTT GACCGGGGTT TGAGGGTACC TAGTGTTGGG GTGGTACTTG

21701 CTTATTACCG GGGTACCCAA CTCCATGCTC AACAGTCCCC AGGTACAGCC  
GAATAATGGC CCCATGGGTT GAGGTACGAG TTGTCAGGGG TCCATGTCGG

21751 CACCCTGCGT CGCAACCAGG AACAGCTCTA CAGCTTCCTG GAGCGCCACT  
GTGGGACGCA GCGTTGGTCC TTGTCGAGAT GTCGAAGGAC CTCGCGGTGA

21801 CGCCCTACTT CCGCAGCCAC AGTGCGCAGA TTAGGAGCGC CACTTCTTTT  
GCGGGATGAA GCGGTCGGTG TCACGCGTCT AATCCTCGCG GTGAAGAAAA

21851 TGTCATTGA AAAACATGTA AAAATAATGT ACTAGAGACA CTTTCAATAA  
ACAGTGAAC TTTTGTACAT TTTTATTACA TGATCTCTGT GAAAGTTATT

21901 AGGCAAATGC TTTTATTTGT AACTCTCGG GTGATTATTT ACCCCCACCC  
TCCGTTTACG AAAATAAACA TGTGAGAGCC CACTAATAAA TGGGGGTGGG

21951 TTGCCGTCTG CGCCGTTTAA AAATCAAAGG GGTTCGCGG CGCATCGCTA  
AACGGCAGAC GCGGCAAATT TTTAGTTTCC CCAAGACGGC GCGTAGCGAT

22001 TGCGCCACTG GCAGGGACAC GTTGCGATAC TGGTGTTTAG TGCTCCACTT  
ACGCGGTGAC CGTCCCTGTG CAACGCTATG ACCACAAATC ACGAGGTGAA

FIG.9A-26

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22051 AAACTCAGGC ACAACCATCC GCGGCAGCTC GGTGAAGTTT TCACTCCACA  
 TTTGAGTCCG TGTGGTAGG CGCCGTCGAG CCACTTCAAA AGTGAGGTGT  
 22101 GGCTGCGCAC CATCACCAAC GCGTTTAGCA GGTGGGGCGC CGATATCTTG  
 CCGACGCGTG GTAGTGGTTG CGCAAATCGT CCAGCCCGCG GCTATAGAAC  
 22151 AAGTCGCAGT TGGGGCCTCC GCCCTGCGCG CGCGAGTTGC GATACACAGG  
 TTCAGCGTCA ACCCCGGAGG CGGGACGCGC GCGCTCAACG CTATGTGTCC  
 22201 GTTGCAGCAC TGGAACACTA TCAGCGCCGG GTGGTGCACG CTGGCCAGCA  
 CAACGTCGTG ACCTTGTGAT AGTCGCGGCC CACCACGTGC GACCGGTCGT  
 22251 CGCTCTTGTC GGAGATCAGA TCCGCGTCCA GGTCTCCGC GTTGCTCAGG  
 GCGAGAACAG CCTCTAGTCT AGGCGCAGGT CCAGGAGGCG CAACGAGTCC  
 22301 GCGAACGGAG TCAACTTTGG TAGCTGCCTT CCCAAAAAGG GCGCGTGCCC  
 CGCTTGCCCTC AGTTGAAACC ATCGACGGAA GGGTTTTTCC CGCGCACGGG  
 22351 AGGCTTTGAG TTGCACTCGC ACCGTAGTGG CATCAAAAGG TGACCGTGCC  
 TCCGAAACTC AACGTGAGCG TGGCATCACC GTAGTTTTCC ACTGGCACGG  
 22401 CGGTCTGGGC GTTAGGATAC AGCGCCTGCA TAAAAGCCTT GATCTGCTTA  
 GCCAGACCCG CAATCCTATG TCGCGGACGT ATTTTCGGAA CTAGACGAAT  
 22451 AAAGCCACCT GAGCCTTTGC GCCTTCAGAG AAGAACATGC CGCAAGACTT  
 TTTCGGTGGA CTCGGAAACG CGGAAGTCTC TTCTTGACG GCGTTCTGAA  
 22501 GCCGGAAAAC TGATTGGCCG GACAGGCCGC GTCGTGCACG CAGCACCTTG  
 CGGCCTTTTG ACTAACCGGC CTGTCCGGCG CAGCACGTGC GTCGTGGAAC  
 22551 CGTCGGTGTT GGAGATCTGC ACCACATTTT GGCCCCACCG GTTCTTCACG  
 GCAGCCACAA CCTCTAGACG TGGTGTAAAG CCGGGGTGGC CAAGAAAGTG  
 22601 ATCTTGGCCT TGCTAGACTG CTCCTTCAGC GCGCGCTGCC CGTTTTCGCT  
 TAGAACCGGA ACGATCTGAC GAGGAAGTCG CGCGCGACGG GCAAAAGCGA  
 22651 CGTCACATCC ATTTCAATCA CGTGCTCCTT ATTTATCATA ATGCTTCCGT  
 GCAGTGTAGG TAAAGTTAGT GCACGAGGAA TAAATAGTAT TACGAAGGCA  
 22701 GTAGACACTT AAGCTCGCCT TCGATCTCAG CGCAGCGGTG CAGCCACAAC  
 CATCTGTGAA TTCGAGCGGA AGCTAGAGTC GCGTCGCCAC GTCGGTGTG  
 22751 GCGCAGCCCG TGGGCTCGTG ATGCTTGTAG GTCACCTCTG CAAACGACTG  
 CGCGTCGGGC ACCCGAGCAC TACGAACATC CAGTGGAGAC GTTTGCTGAC  
 22801 CAGGTACGCC TGCAGGAATC GCCCCATCAT CGTCACAAAG GTCTTGTTGC  
 GTCCATGCGG ACGTCCTTAG CGGGGTAGTA GCAGTGTTTC CAGAACAACG  
 22851 TGGTGAAGGT CAGCTGCAAC CCGCGGTGCT CTCGTTCAG CCAGGTCTTG  
 ACCACTTCCA GTCGACGTTG GGCGCCACGA GGAGCAAGTC GTTCCAGAAC

FIG.9A-27

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22901	CATACGGCCG GTATGCCGGC	CCAGAGCTTC GGTCTCGAAG	CAC TTGGTCA GTGAACCAGT	GGCAGTAGTT CCGTCATCAA	TGAAGTTCGC ACTTCAAGCG
22951	CTTTAGATCG GAAATCTAGC	TTATCCACGT AATAGGTGCA	GGTACTTGTC CCATGAACAG	CATCAGCGCG GTAGTCGCGC	CGCGCAGCCT GCGCGTCGGA
23001	CCATGCCCTT GGTACGGGAA	CTCCCACGCA GAGGGTGCGT	GACACGATCG CTGTGCTAGC	GCACACTCAG CGTGTGAGTC	CGGGTTCATC GCCCAAGTAG
23051	ACCGTAATTT TGGCATTAAA	CAC TTTCCGC GTGAAAGGCG	TTCGCTGGGC AAGCGACCCG	TCTTCCTCTT AGAAGGAGAA	CCTCTTGCGT GGAGAACGCA
23101	CCGCATACCA GGCGTATGGT	CGCGCCACTG GCGCGGTGAC	GGTCGTCTTC CCAGCAGAAG	ATTCAGCCGC TAAGTCGGCG	CGCACTGTGC GCGTGACACG
23151	GCTTACCTCC CGAATGGAGG	TTTGCCATGC AAACGGTACG	TTGATTAGCA AACTAATCGT	CCGGTGGGTT GGCCACCCAA	GCTGAAACCC CGACTTTGGG
23201	ACCATTTGTA TGGTAAACAT	GCGCCACATC CGCGGTGTAG	TTCTCTTTCT AAGAGAAAAG	TCCTCGCTGT AGGAGCGACA	CCACGATTAC GGTGCTAATG
23251	CTCTGGTGAT GAGACCACTA	GGCGGGCGCT CCGCCC GCGA	CGGGCTTGGG GCCCCAACCC	AGAAGGGCGC TCTTCCCGCG	TTCTTTTTCT AAGAAAAAGA
23301	TCTTGGGCGC AGAACCCGCG	AATGGCCAAA TTACCGGTTT	TCCGCCGCCG AGGCGGCGGC	AGGTCGATGG TCCAGCTACC	CCGCGGGCTG GGCGCCCGAC
23351	GGTGTGCGCG CCACACGCGC	GCACCAGCGC CGTGGTCGCG	GTCTTG TGAT CAGAACTACTA	GAGTCTTCCT CTCAGAAGGA	CGTCCTCGGA GCAGGAGCCT
23401	CTCGATACGC GAGCTATGCG	CGCCTCATCC GCGGAGTAGG	GCTTTTTTTGG CGAAAAAACC	GGGCGCCCGG CCCGCGGGCC	GGAGGCGGCG CCTCCGCCGC
23451	GCGACGGGGA CGCTGCCCTT	CGGGGACGAC GCCCCTGCTG	ACGTCCTCCA TGCAGGAGGT	TGGTTGGGGG ACCAACCCCC	ACGTCGCGCC TGCAGCGCGG
23501	GCACCGCGTC CGTGGCGCAG	CGCGCTCGGG GCGCGAGCCC	GGTGGTTTCG CCACCAAAGC	CGCTGCTCCT GCGACGAGGA	CTTCCCGACT GAAGGGCTGA
23551	GGCCATTTCC CCGGTAAAGG	TTCTCCTATA AAGAGGATAT	GGCAGAAAAA CCGTCTTTTT	GATCATGGAG CTAGTACCTC	TCAGTCGAGA AGTCAGCTCT
23601	AGAAGGACAG TCTTCCTGTC	CCTAACCGCC GGATTGGCGG	CCCTCTGAGT GGGAGACTCA	TCGCCACCAC AGCGGTGGTG	CGCCTCCACC GCGGAGGTGG
23651	GATGCCGCCA CTACGGCGGT	ACGCGCCTAC TGCGCGGATG	CACCTTCCCC GTGGAAGGGG	GTCGAGGCAC CAGCTCCGTG	CCCCGCTTGA GGGGCGAACT
23701	GGAGGAGGAA CCTCCTCCTT	GTGATTATCG CACTAATAGC	AGCAGGACCC TCGTCCTGGG	AGGTTTTGTA TCCAAAACAT	AGCGAAGACG TCGCTTCTGC

FIG.9A-28



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23751 ACGAGGACCG CTCAGTACCA ACAGAGGATA AAAAGCAAGA CCAGGACAAC  
TGCTCCTGGC GAGTCATGGT TGTCTCCTAT TTTTCGTTCT GGTCTGTGTG

23801 GCAGAGGCAA ACGAGGAACA AGTCGGGCGG GGGGACGAAA GGCATGGCGA  
CGTCTCCGTT TGCTCCTTGT TCAGCCCGCC CCCCTGCTTT CCGTACCGCT

23851 CTACCTAGAT GTGGGAGACG ACGTGCTGTT GAAGCATCTG CAGCGCCAGT  
GATGGATCTA CACCCTCTGC TGCACGACAA CTTCGTAGAC GTCGCGGTCA

23901 GCGCCATTAT CTGCGACGCG TTGCAAGAGC GCAGCGATGT GCCCCTCGCC  
CGCGGTAATA GACGCTGCGC AACGTTCTCG CGTCGCTACA CGGGGAGCGG

23951 ATAGCGGATG TCAGCCTTGC CTACGAACGC CACCTATTCT CACCGCGCGT  
TATCGCCTAC AGTCGGAACG GATGCTTGCG GTGGATAAGA GTGGCGCGCA

24001 ACCCCCCAAA CGCCAAGAAA ACGGCACATG CGAGCCCAAC CCGCGCCTCA  
TGGGGGGTTT GCGGTTCTTT TGCCGTGTAC GCTCGGGTTG GGC GCGGAGT

24051 ACTTCTACCC CGTATTTGCC GTGCCAGAGG TGCTTGCCAC CTATCACATC  
TGAAGATGGG GCATAAACGG CACGGTCTCC ACGAACGGTG GATAGTGTAG

24101 TTTTTCCAAA ACTGCAAGAT ACCCCTATCC TGCCGTGCCA ACCGCAGCCG  
AAAAAGGTTT TGACGTTCTA TGGGGATAGG ACGGCACGGT TGGCGTCGGC

24151 AGCGGACAAG CAGCTGGCCT TGCGGCAGGG CGCTGTCATA CCTGATATCG  
TCGCTGTTC GTCGACCGGA ACGCCGTCCC GCGACAGTAT GGA CTATAGC

24201 CCTCGCTCAA CGAAGTGCCA AAAATCTTTG AGGGTCTTGG ACGCGACGAG  
GGAGCGAGTT GCTTCACGGT TTTTAGAAAC TCCCAGAAC TGCCTGCTC

24251 AAGCGCGCGG CAAACGCTCT GCAACAGGAA AACAGCGAAA ATGAAAGTCA  
TTCGCGCGCC GTTTGCGAGA CGTTGTCCTT TTGTCGCTTT TACTTTAGT

24301 CTCTGGAGTG TTGGTGGAAC TCGAGGGTGA CAACGCGCGC CTAGCCGTAC  
GAGACCTCAC AACCACCTTG AGCTCCCACT GTTGCGCGCG GATCGGCATG

24351 TAAACGCAG CATCGAGGTC ACCCACTTTG CCTACCCGGC ACTTAACCTA  
ATTTTGCGTC GTAGCTCCAG TGGGTGAAAC GGATGGGCCG TGAATTGGAT

24401 CCCCCCAAGG TCATGAGCAC AGTCATGAGT GAGCTGATCG TCGCCGTGC  
GGGGGGTTCC AGTACTCGTG TCAGTACTCA CTCGACTAGC ACGCGGCACG

24451 GCAGCCCCTG GAGAGGGATG CAAATTTGCA AGAACAAACA GAGGAGGGCC  
CGTCGGGGAC CTCTCCCTAC GTTTAAACGT TCTTGTGTTG CTCTCCCGG

24501 TACCCGCACT TGGCGACGAG CAGCTAGCGC GCTGGCTTCA AACGCGCGAG  
ATGGGCGTCA ACCGCTGCTC GTCGATCGCG CGACCGAAGT TTGCGCGCTC

24551 CCTGCCGACT TGGAGGAGCG ACGCAAATA ATGATGGCCG CAGTGCTCGT  
GGACGGCTGA ACCTCCTCGC TGCGTTTGAT TACTACCGGC GTCACGAGCA

FIG.9A-29

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24601	TACCGTGGAG ATGGCACCTC	CTTGAGTGCA GAACTCACGT	TGCAGCGGTT ACGTGCGCAA	CTTTGCTGAC GAAACGACTG	CCGGAGATGC GGCCTCTACG
24651	AGCGCAAGCT TCGCGTTCGA	AGAGGAAACA TCTCCTTTGT	TTGCACTACA AACGTGATGT	CCTTTTCGACA GGAAAGCTGT	GGGCTACGTA CCCGATGCAT
24701	CGCCAGGCCT GCGGTCCGGA	GCAAGATCTC CGTTCTAGAG	CAACGTGGAG GTTGCACCTC	CTCTGCAACC GAGACGTTGG	TGGTCTCCTA ACCAGAGGAT
24751	CCTTGGAATT GGAACCTTAA	TTGCACGAAA AACGTGCTTT	ACCGCCTTGG TGGCGGAACC	GCAAAACGTG CGTTTTGCAC	CTTCATTCCA GAAGTAAGGT
24801	CGCTCAAGGG GCGAGTTCCC	CGAGGCGCGC GCTCCGCGCG	CGCGACTACG GCGCTGATGC	TCCGCGACTG AGGCGCTGAC	CGTTTACTTA GCAAATGAAT
24851	TTTCTATGCT AAAGATACGA	ACACCTGGCA TGTGGACCGT	GACGGCCATG CTGCCGGTAC	GGCGTTTGGC CCGCAAACCG	AGCAGTGCTT TCGTCACGAA
24901	GGAGGAGTGC CCTCCTCACG	AACCTCAAGG TTGGAGTTCC	AGCTGCAGAA TCGACGTCTT	ACTGCTAAAG TGACGATTTC	CAAAACTTGA GTTTTGAACT
24951	AGGACCTATG TCCTGGATAC	GACGGCCTTC CTGCCGGAAG	AACGAGCGCT TTGCTCGCGA	CCGTGGCCGC GGCACCGGCG	GCACCTGGCG CGTGGACCGC
25001	GACATCATTT CTGTAGTAAA	TCCCCGAACG AGGGGCTTGC	CCTGCTTAAA GGACGAATTT	ACCCTGCAAC TGGGACGTTG	AGGGTCTGCC TCCCAGACGG
25051	AGACTTCACC TCTGAAGTGG	AGTCAAAGCA TCAGTTTCGT	TGTTGCAGAA ACAACGTCTT	CTTTAGGAAC GAAATCCTTG	TTTATCCTAG AAATAGGATC
25101	AGCGCTCAGG TCGCGAGTCC	AATCTTGCCC TTAGAACGGG	GCCACCTGCT CGGTGGACGA	GTGCACTTCC CACGTGAAGG	TAGCGACTTT ATCGCTGAAA
25151	GTGCCCATT CACGGGTAAT	AGTACCGCGA TCATGGCGCT	ATGCCCTCCG TACGGGAGGC	CCGCTTTGGG GGCGAAACCC	GCCACTGCTA CGGTGACGAT
25201	CCTTCTGCAG GGAAGACGTC	CTAGCCAACT GATCGGTTGA	ACCTTGCCCTA TGGAACGGAT	CCACTCTGAC GGTGAGACTG	ATAATGGAAG TATTACCTTC
25251	ACGTGAGCGG TGCACTCGCC	TGACGGTCTA ACTGCCAGAT	CTGGAGTGTC GACCTCACAG	ACTGTCGCTG TGACAGCGAC	CAACCTATGC GTTGGATACG
25301	ACCCCGCACC TGGGGCGTGG	GCTCCCTGGT CGAGGGACCA	TTGCAATTCTG AACGTTAAGC	CAGCTGCTTA GTCGACGAAT	ACGAAAGTCA TGCTTTTCAGT
25351	AATTATCGGT TTAATAGCCA	ACCTTTGAGC TGGAAACTCG	TGCAGGGTCC ACGTCCCAGG	CTCGCCTGAC GAGCGGACTG	GAAAAGTCCG CTTTTCAGGC
25401	CGGCTCCGGG GCCGAGGCC	GTTGAAACTC CAACTTTGAG	ACTCCGGGGC TGAGGCCCCG	TGTGGACGTC ACACCTGCAG	GGCTTACCTT CCGAATGGAA

FIG.9A-30

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25451	CGCAAATTTG GCGTTTAAAC	TACCTGAGGA ATGGACTCCT	CTACCACGCC GATGGTGCGG	CACGAGATTA GTGCTCTAAT	GGTTCTACGA CCAAGATGCT
25501	AGACCAATCC TCTGGTTAGG	CGCCCGCCTA GCGGGCGGAT	ATGCGGAGCT TACGCCTCGA	TACCGCCTGC ATGGCGGACG	GTCATTACCC CAGTAATGGG
25551	AGGGCCACAT TCCCGGTGTA	TCTTGGCCAA AGAACCGGTT	TTGCAAGCCA AACGTTCTGGT	TCAACAAAGC AGTTGTTTCG	CCGCCAAGAG GGCGGTTCTC
25601	TTTCTGCTAC AAAGACGATG	GAAAGGGACG CTTTCCTGCT	GGGGGTTTAC CCCCCAAATG	TTGGACCCCC AACCTGGGGG	AGTCCGGCGA TCAGGCCGCT
25651	GGAGCTCAAC CCTCGAGTTG	CCAATCCCCC GGTTAGGGGG	CGCCGCCGCA GCGGCGGCGT	GCCCTATCAG CGGGATAGTC	CAGCAGCCGC GTCGTCGGCG
25701	GGGCCCTTGC CCCGGGAACG	TTCCCAGGAT AAGGGTCCTA	GGCACCCAAA CCGTGGGTTT	AAGAAGCTGC TTCTTCGACG	AGCTGCCGCC TCGACGGCGG
25751	GCCACCCACG CGGTGGGTGC	GACGAGGAGG CTGCTCCTCC	AATACTGGGA TTATGACCCT	CAGTCAGGCA GTCAGTCCGT	GAGGAGGTTT CTCCTCCAAA
25801	TGGACGAGGA ACCTGCTCCT	GGAGGAGGAC CCTCCTCCTG	ATGATGGAAG TACTACCTTC	ACTGGGAGAG TGACCCTCTC	CCTAGACGAG GGATCTGCTC
25851	GAAGCTTCCG CTTCGAAGGC	AGGTCAAGAA TCCAGCTTCT	GGTGTGAGAC CCACAGTCTG	GAAACACCGT CTTTGTGGCA	CACCCTCGGT GTGGGAGCCA
25901	CGCATTCCCC GCGTAAGGGG	TCGCCGGCGC AGCGGCCGCG	CCCAGAAATC GGGTCTTTAG	GGCAACCGGT CCGTTGGCCA	TCCAGCATGG AGGTCGTACC
25951	CTACAACCTC GATGTTGGAG	CGCTCCTCAG GCGAGGAGTC	GCGCCGCCGG CGCGCGGCC	CACTGCCCCT GTGACGGGCA	TCGCCGACCC AGCGGCTGGG
26001	AACCGTAGAT TTGGCATCTA	GGGACACCAC CCCTGTGGTG	TGGAACCAGG ACCTTGGTCC	GCCGGTAAGT CGGCCATTCA	CCAAGCAGCC GGTTCGTGCG
26051	GCCGCCGTTA CGGCGGCAAT	GCCCAAGAGC CGGGTTCTCG	AACAACAGCG TTGTTGTCGC	CCAAGGCTAC GGTTCCGATG	CGCTCATGGC GCGAGTACCG
26101	GCGGGCACAA CGCCCCGTGT	GAACGCCATA CTTGCGGTAT	GTTGCTTGCT CAACGAACGA	TGCAAGACTG ACGTTCTGAC	TGGGGGCAAC ACCCCCGTG
26151	ATCTCCTTCG TAGAGGAAGC	CCCGCCGCTT GGGCGGCGAA	TCTTCTCTAC AGAAGAGATG	CATCACGGCG GTAGTGCCGC	TGGCCTTCCC ACCGGAAGGG
26201	CCGTAACATC GGCATTGTAG	CTGCATTACT GACGTAATGA	ACCGTCATCT TGGCAGTAGA	CTACAGCCCA GATGTCGGGT	TACTGCACCG ATGACGTGGC
26251	GCGGCAGCGG CGCCGTCGCC	CAGCAACAGC GTCGTTGTCG	AGCGGCCACA TCGCCGGTGT	CAGAAGCAAA GTCTTCGTTT	GGCGACCGGA CCGCTGGCCT

FIG.9A-31

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26301	TAGCAAGACT ATCGTTCTGA	CTGACAAAGC GACTGTTTCG	CCAAGAAATC GGTTCCTTTAG	CACAGCGGCG GTGTCGCCGC	GCAGCAGCAG CGTCGTCGTC
26351	GAGGAGGAGC CTCCTCCTCG	GCTGCGTCTG CGACGCAGAC	GCGCCCAACG CGCGGGTTGC	AACCCGTATC TTGGGCATAG	GACCCGCGAG CTGGGCGCTC
26401	CTTAGAAACA GAATCTTTGT	GGATTTTTCC CCTAAAAAGG	CACTCTGTAT GTGAGACATA	GCTATATTTT CGATATAAAG	AACAGAGCAG TTGTCTCGTC
26451	GGGCCAAGAA CCCGGTTCTT	CAAGAGCTGA GTTCTCGACT	AAATAAAAAA TTTATTTTTT	CAGGTCTCTG GTCCAGAGAC	CGATCCCTCA GCTAGGGAGT
26501	CCCGCAGCTG GGGCGTCGAC	CCTGTATCAC GGACATAGTG	AAAAGCGAAG TTTTCGCTTC	ATCAGCTTCG TAGTCGAAGC	GCGCACGCTG CGCGTGCGAC
26551	GAAGACGCGG CTTCTGCGCC	AGGCTCTCTT TCCGAGAGAA	CAGTAAATAC GTCATTTATG	TGCGCGCTGA ACGCGCGACT	CTCTTAAGGA GAGAATTCCT
26601	CTAGTTTCGC GATCAAAGCG	GCCCTTTCTC CGGGAAAGAG	AAATTTAAGC TTTAAATTCG	GCGAAACTA CGCTTTTGAT	CGTCATCTCC GCAGTAGAGG
26651	AGCGGCCACA TCGCCGGTGT	CCCGGCGCCA GGGCCGCGGT	GCACCTGTTG CGTGGACAAC	TCAGCGCCAT AGTCGCGGTA	TATGAGCAAG ATACTCGTTC
26701	GAAATTCCCA CTTTAAGGGT	CGCCCTACAT GCGGGATGTA	GTGGAGTTAC CACCTCAATG	CAGCCACAAA GTCGGTGTTT	TGGGACTTGC ACCCTGAACG
26751	GGCTGGAGCT CCGACCTCGA	GCCCAAGACT CGGGTTCTGA	ACTCAACCCG TGAGTTGGGC	AATAAACTAC TTATTTGATG	ATGAGCGCGG TACTCGCGCC
26801	GACCCACAT CTGGGGTGTA	GATATCCCGG CTATAGGGCC	GTCAACGGAA CAGTTGCCTT	TACGCGCCCA ATGCGCGGGT	CCGAAACCGA GGCTTTGGCT
26851	ATTCTCCTGG TAAGAGGACC	AACAGGCGGC TTGTCCGCCG	TATTACCACC ATAATGGTGG	ACACCTCGTA TGTGGAGCAT	ATAACCTTAA TATTGGAATT
26901	TCCCCGTAGT AGGGGCATCA	TGGCCCGCTG ACCGGGCGAC	CCCTGGTGTA GGGACCACAT	CCAGGAAAGT GGTCCTTTCA	CCCGCTCCCA GGGCGAGGGT
26951	CCACTGTGGT GGTGACACCA	ACTTCCCAGA TGAAGGGTCT	GACGCCCAGG CTGCGGGTCC	CCGAAGTTCA GGCTTCAAGT	GATGACTAAC CTACTGATTG
27001	TCAGGGGCGC AGTCCCCGCG	AGCTTGCGGG TCGAACGCC	CGGCTTTCGT GCCGAAAGCA	CACAGGGTGC GTGTCCACG	GGTCGCCCCG CCAGCGGGCC
27051	GCAGGGTATA CGTCCCATAT	ACTCACCTGA TGAGTGGACT	CAATCAGAGG GTTAGTCTCC	GCGAGGTATT CGCTCCATAA	CAGCTCAACG GTCGAGTTGC
27101	ACGAGTCGGT TGCTCAGCCA	GAGCTCCTCG CTCGAGGAGC	CTTGGTCTCC GAACCAGAGG	GTCCGGACGG CAGGCCTGCC	GACATTTTCAG CTGTAAAGTC

FIG.9A-32

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27151 ATCGGCGGCG CCGGCCGCTC TTCATTCACG CCTCGTCAGG CAATCCTAAC  
 TAGCCGCCGC GGCCGGCGAG AAGTAAGTGC GGAGCAGTCC GTTAGGATTG  
 27201 TCTGCAGACC TCGTCCTCTG AGCCGCGCTC TGGAGGCATT GGAAGTCTGC  
 AGACGTCTGG AGCAGGAGAC TCGGCGCGAG ACCTCCGTAA CCTTGAGACG  
 27251 AATTTATTGA GGAGTTTGTG CCATCGGTCT ACTTTAACCC CTTCTCGGGA  
 TTAAATAACT CCTCAAACAC GGTAGCCAGA TGAAATTGGG GAAGAGCCCT  
 27301 CCTCCCGGCC ACTATCCGGA TCAATTTATT CCTAACTTTG ACGCGGTAAA  
 GGAGGGCCGG TGATAGGCCT AGTTAAATAA GGATTGAAAC TGCGCCATTT  
 27351 GGAAGTGGAGAG GCAGAGCAAC  
 CCTGAGCCGC CTGCCGATGC TGACTTACAA TTCACCTCTC CGTCTCGTTG  
 27401 TGCGCCTGAA ACACCTGGTC CACTGTCGCC GCCACAAGTG CTTTGCCCCG  
 ACGCGGACTT TGTGGACCAG GTGACAGCGG CCGTGTTTAC GAAACGGGCG  
 27451 GACTCCGGTG AGTTTTGCTA CTTTGAATTG CCCGAGGATC ATATCGAGGG  
 CTGAGGCCAC TCAAAACGAT GAAACTTAAC GGGCTCCTAG TATAGCTCCC  
 27501 CCCGGCGCAC GGCCTCCGGC TTACCGCCCA GGGAGAGCTT GCCCGTAGCC  
 GGGCCGCGTG CCGCAGGCCG AATGGCGGGT CCCTCTCGAA CGGGCATCGG  
 27551 TGATTCGGGA GTTTACCCAG CGCCCCCTGC TAGTTGAGCG GGACAGGGGA  
 ACTAAGCCCT CAAATGGGTC GCGGGGGACG ATCAACTCGC CCTGTCCCCT  
 27601 CCCTGTGTTT TCACTGTGAT TTGCAACTGT CCTAACCCCTG GATTACATCA  
 GGGACACAAG AGTGACACTA AACGTTGACA GGATTGGGAC CTAATGTAGT  
 27651 AGATCTTTGT TGCCATCTCT GTGCTGAGTA TAATAAATAC AGAAATTAAC  
 TCTAGAAACA ACGGTAGAGA CACGACTCAT ATTATTTATG TCTTTAATTT  
 27701 ATATACTGGG GCTCCTATCG CCATCCTGTA AACGCCACCG TCTTCACCCG  
 TATATGACCC CGAGGATAGC GGTAGGACAT TTGCGGTGGC AGAAGTGGGC  
 27751 CCCAAGCAAA CCAAGGCGAA CCTTACCTGG TACTTTTAAC ATCTCTCCCT  
 GGGTTCGTTT GGTTCCGCTT GGAATGGACC ATGAAAATTG TAGAGAGGGA  
 27801 CTGTGATTTA CAACAGTTTC AACCCAGACG GAGTGAGTCT ACGAGAGAAC  
 GACACTAAAT GTTGTCAAAG TTGGGTCTGC CTCACTCAGA TGCTCTCTTG  
 27851 CTCTCCGAGC TCAGCTACTC CATCAGAAAA AACACCACCC TCCTTACCTG  
 GAGAGGCTCG AGTCGATGAG GTAGTCTTTT TTGTGGTGGG AGGAATGGAC  
 27901 CCGGGAACGT ACGAGTGCGT CACCGGCCGC TGCACCACAC CTACCGCCTG  
 GGCCCTTGCA TGCTCACGCA GTGGCCGGCG ACGTGGTGTG GATGGCGGAC  
 27951 ACCGTAAACC AGACTTTTTTC CGGACAGACC TCAATAACTC TGTTTACCAG  
 TGGCATTGTT TCTGAAAAAG GCCTGTCTGG AGTTATTGAG ACAAATGGTC

FIG.9A-33

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28001	AACAGGAGGT TTGTCCCTCCA	GAGCTTAGAA CTCGAATCTT	AACCCTTAGG TTGGGAATCC	GTATTAGGCC CATAATCCGG	AAAGGCGCAG TTTCCGCGTC
28051	CTACTGTGGG GATGACACCC	GTTTATGAAC CAAATACTTG	AATTCAAGCA TTAAGTTCGT	ACTCTACGGG TGAGATGCCC	CTATTCTAAT GATAAGATTA
28101	TCAGGTTTCT AGTCCAAAGA	CTAGAATCGG GATCTTAGCC	GGTTGGGGTT CCAACCCCAA	ATTCTCTGTC TAAGAGACAG	TTGTGATTCT AACACTAAGA
28151	CTTTATTCTT GAAATAAGAA	ATACTAACGC TATGATTGCG	TTCTCTGCCT AAGAGACGGA	AAGGCTCGCC TTCCGAGCGG	GCCTGCTGTG CGGACGACAC
28201	TGCACATTTG ACGTGTAAAC	CATTTATTGT GTAAATAACA	CAGCTTTTTA GTCGAAAAT	AACGCTGGGG TTGCGACCCC	TCGCCACCCA AGCGGTGGGT
28251	AGATGATTAG TCTACTAATC	GTACATAATC CATGTATTAG	CTAGGTTTAC GATCCAAATG	TCACCCTTGC AGTGGGAACG	GTCAGCCCAC CAGTCGGGTG
28301	GGTACCACCC CCATGGTGGG	AAAAGGTGGA TTTTCCACCT	TTTTAAGGAG AAAATTCCTC	CCAGCCTGTA GGTCGGACAT	ATGTTACATT TACAATGTAA
28351	CGCAGCTGAA GCGTCGACTT	GCTAATGAGT CGATTACTCA	GCACCACTCT CGTGGTGAGA	TATAAAATGC ATATTTTACG	ACCACAGAAC TGGTGTCTTG
28401	ATGAAAAGCT TACTTTTTCGA	GCTTATTCGC CGAATAAGCG	CACAAAAACA GTGTTTTTGT	AAATTGGCAA TTTAACCGTT	GTATGCTGTT CATACGACAA
28451	TATGCTATTT ATACGATAAA	GGCAGCCAGG CCGTCGGTCC	TGACACTACA ACTGTGATGT	GAGTATAATG CTCATATTAC	TTACAGTTTT AATGTCAAAA
28501	CCAGGGTAAA GGTCCCATT	AGTCATAAAA TCAGTATTTT	CTTTTATGTA GAAAATACAT	TACTTTTCCA ATGAAAAGGT	TTTTATGAAA AAAATACTTT
28551	TGTGCGACAT ACACGCTGTA	TACCATGTAC ATGGTACATG	ATGAGCAAAC TACTCGTTTG	AGTATAAGTT TCATATTCAA	GTGGCCCCCA CACCGGGGGT
28601	CAAAATTGTG GTTTTAACAC	TGGAAAACAC ACCTTTTGTG	TGGCACTTTC ACCGTGAAAG	TGCTGCACTG ACGACGTGAC	CTATGCTAAT GATACGATTA
28651	TACAGTGCTC ATGTCACGAG	GCTTTGGTCT CGAAACCAGA	GTACCCTACT CATGGGATGA	CTATATTAAA GATATAATTT	TACAAAAGCA ATGTTTTTCGT
28701	GACGCAGCTT CTGCGTCGAA	TATTGAGGAA ATAACTCCTT	AAGAAAATGC TTCTTTTACG	CTTAATTTAC GAATTAAATG	TAAGTTACAA ATTCAATGTT
28751	AGCTAATGTC TCGATTACAG	ACCACTAACT TGGTGATTGA	GCTTTACTCG CGAAATGAGC	CTGCTTGCAA GACGAACGTT	AACAAATTCA TTGTTTAAGT
28801	AAAAGTTAGC TTTTCAATCG	ATTATAATTA TAATATTAAT	GAATAGGATT CTTATCCTAA	TAAACCCCCC ATTTGGGGGG	GGTCATTTCC CCAGTAAAGG

FIG.9A-34

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28851	TGCTCAATAC	CATTCCCCTG	AACAATTGAC	TCTATGTGGG	ATATGCTCCA
	ACGAGTTATG	GTAAGGGGAC	TTGTAACTG	AGATACACCC	TATACGAGGT
28901	GCGCTACAAC	CTTGAAGTCA	GGCTTCCTGG	ATGTCAGCAT	CTGACTTTGG
	CGCGATGTTG	GAAC TTCAGT	CCGAAGGACC	TACAGTCGTA	GACTGAAACC
28951	CCAGCACCTG	TCCCGCGGAT	TTGTTCCAGT	CCAAC TACAG	CGACCCACCC
	GGTCGTGGAC	AGGGCGCCTA	AACAAGGTCA	GGTTGATGTC	GCTGGGTGGG
29001	TAACAGAGAT	GACCAACACA	ACCAACGCGG	CCGCCGCTAC	CGGACTTACA
	ATTGTCTCTA	CTGGTTGTGT	TGGTTGCGCC	GGCGGCGATG	GCCTGAATGT
29051	TCTACCACAA	ATACACCCCA	AGTTTCTGCC	TTTGTCAATA	ACTGGGATAA
	AGATGGTGTT	TATGTGGGGT	TCAAAGACGG	AAACAGTTAT	TGACCCTATT
29101	CTTGGGCATG	TGGTGGTTCT	CCATAGCGCT	TATGTTTGTA	TGCCTTATTA
	GAACCCGTAC	ACCACCAAGA	GGTATCGCGA	ATACAAACAT	ACGGAATAAT
29151	TTATGTGGCT	CATCTGCTGC	CTAAAGCGCA	AACGCGCCCG	ACCACCCATC
	AATACACCGA	GTAGACGACG	GATTCGCGT	TTGCGCGGGC	TGGTGGGTAG
29201	TATAGTCCCA	TCATTGTGCT	ACACCCAAAC	AATGATGGAA	TCCATAGATT
	ATATCAGGGT	AGTAACACGA	TGTGGGTTTG	TTACTACCTT	AGGTATCTAA
29251	GGACGGACTG	AAACACATGT	TCTTTTCTCT	TACAGTATGA	TTAAATGAGA
	CCTGCCTGAC	TTTGTGTACA	AGAAAAGAGA	ATGTCATACT	AATTTACTCT
29301	CATGATTCCT	CGAGTTTTTA	TATTACTGAC	CCTTGTTGCG	CTTTTTTGTC
	GTAATAAGGA	GCTCAAAAAT	ATAATGACTG	GGAACAACGC	GAAAAAACAC
29351	CGTGCTCCAC	ATTGGCTGCG	GTTTCTCACA	TCGAAGTAGA	CTGCATTCCA
	GCACGAGGTG	TAACCGACGC	CAAAGAGTGT	AGCTTCATCT	GACGTAAGGT
29401	GCCTTCACAG	TCTATTTGCT	TTACGGATTT	GTCACCCTCA	CGCTCATCTG
	CGGAAGTGTC	AGATAAACGA	AATGCCTAAA	CAGTGGGAGT	GCGAGTAGAC
29451	CAGCCTCATC	ACTGTGGTCA	TCGCCTTTAT	CCAGTGCAAT	GACTGGGTCT
	GTCGGAGTAG	TGACACCAGT	AGCGGAAATA	GGTCACGTAA	CTGACCCAGA
29501	GTGTGCGCTT	TGCATATCTC	AGACACCATC	CCCAGTACAG	GGACAGGACT
	CACACGCGAA	ACGTATAGAG	TCTGTGGTAG	GGGTCATGTC	CCTGTCCTGA
29551	ATAGCTGAGC	TTCTTAGAAT	TCTTTAATTA	TGAAATTTAC	TGTGACTTTT
	TATCGACTCG	AAGAATCTTA	AGAAATTAAT	ACTTTAAATG	ACACTGAAAA
29601	CTGCTGATTA	TTTGCACCTT	ATCTGCGTTT	TGTTCCCCGA	CCTCCAAGCC
	GACGACTAAT	AAACGTGGGA	TAGACGCAA	ACAAGGGGCT	GGAGGTTTCG
29651	TCAAAGACAT	ATATCATGCA	GATTCACTCG	TATATGGAAT	ATTCCAAGTT
	AGTTTCTGTA	TATAGTACGT	CTAAGTGAGC	ATATACCTTA	TAAGGTTCAA

FIG.9A-35

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29701	GCTACAATGA CGATGTTACT	AAAAAGCGAT TTTTTCGCTA	CTTTCCGAAG GAAAGGCTTC	CCTGGTTATA GGACCAATAT	TGCAATCATC ACGTTAGTAG
29751	TCTGTTATGG AGACAATACC	TGTTCTGCAG ACAAGACGTC	TACCATCTTA ATGGTAGAAT	GCCCTAGCTA CGGGATCGAT	TATATCCCTA ATATAGGGAT
29801	CCTTGACATT GGAACGTAA	GGCTGGAACG CCGACCTTGC	CAATAGATGC GTTATCTACG	CATGAACCAC GTACTTGGTG	CCAACTTTCC GGTTGAAAGG
29851	CCGCGCCCCG GGCGCGGGCG	TATGCTTCCA ATACGAAGGT	CTGCAACAAG GACGTTGTTC	TTGTTGCCGG AACAACGGCC	CGGCTTTGTC GCCGAAACAG
29901	CCAGCCAATC GGTCGGTTAG	AGCCTCGCCC TCGGAGCGGG	ACCTTCTCCC TGAAGAGGG	ACCCCCACTG TGGGGGTGAC	AAATCAGCTA TTTAGTCGAT
29951	CTTTAATCTA GAAATTAGAT	ACAGGAGGAG TGTCCTCCTC	ATGACTGACA TACTGACTGT	CCCTAGATCT GGGATCTAGA	AGAAATGGAC TCTTTACCTG
30001	GGAATTATTA CCTTAATAAT	CAGAGCAGCG GTCTCGTCGC	CCTGCTAGAA GGACGATCTT	AGACGCAGGG TCTGCGTCCC	CAGCGGCCGA GTCGCCGGCT
30051	GCAACAGCGC CGTTGTCGCG	ATGAATCAAG TACTTAGTTC	AGCTCCAAGA TCGAGGTTCT	CATGGTTAAC GTACCAATTG	TTGCACCAAGT AACGTGGTCA
30101	GCAAAAGGGG CGTTTTCCCC	TATCTTTTGT ATAGAAAACA	CTCGTAAAGC GAGCATTTTCG	AGGCCAAAAGT TCCGGTTTCA	CACCTACGAC GTGGATGCTG
30151	AGTAATACCA TCATTATGGT	CCGGACACCG GGCCTGTGGC	CCTTAGCTAC GGAATCGATG	AAGTTGCCAA TTCAACGGTT	CCAAGCGTCA GGTTCGCACT
30201	GAAATTGGTG CTTTAACCAC	GTCATGGTGG CAGTACCACC	GAGAAAAGCC CTCTTTTCGG	CATTACCATA GTAATGGTAT	ACTCAGCACT TGAGTCGTGA
30251	CGGTAGAAAC GCCATCTTTG	CGAAGGCTGC GCTTCCGACG	ATCACTCAC TAAGTGAGTG	CTTGTC AAGG GAACAGTTCC	ACCTGAGGAT TGGACTCCTA
30301	CTCTGCACCC GAGACGTGGG	TTATTAAGAC AATAATTCTG	CCTGTGCGGT GGACACGCCA	CTCAAAGATC GAGTTTCTAG	TTATTCCCTT AATAAGGGAA
30351	TAACTAATAA ATTGATTATT	AAAAAAATAA TTTTTTTATT	TAAAGCATCA ATTTTCGTAGT	CTTACTTAAA GAATGAATTT	ATCAGTTAGC TAGTCAATCG
30401	AAATTTCTGT TTTAAAGACA	CCAGTTTATT GGTCAAATAA	CAGCAGCACC GTCGTCGTGG	TCCTTGCCCT AGGAACGGGA	CCTCCCAGCT GGAGGGTCGA
30451	CTGGTATTGC GACCATAACG	AGCTTCCTCC TCGAAGGAGG	TGGCTGCAAA ACCGACGTTT	CTTTCTCCAC GAAAGAGGTG	AATCTAAATG TTAGATTTAC
30501	GAATGTCAGT CTTACAGTCA	TTCCTCCTGT AAGGAGGACA	TCCTGTCCAT AGGACAGGTA	CCGCACCCAC GGCGTGGGTG	TATCTTCATG ATAGAAGTAC

FIG.9A-36



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30551 TTGTTGCAGA TGAAGCGCGC AAGACCGTCT GAAGATACCT TCAACCCCGT  
 AACAACGTCT ACTTCGCGCG TTCTGGCAGA CTTCTATGGA AGTTGGGGCA  
 30601 GTATCCATAT GACACGGAAA CCGGTCCTCC AACTGTGCCT TTTCTTACTC  
 CATAGGTATA CTGTGCCTTT GGCCAGGAGG TTGACACGGA AAAGAATGAG  
 30651 CTCCCTTTGT ATCCCCCAAT GGGTTTCAAG AGAGTCCCCC TGGGGTACTC  
 GAGGGAAACA TAGGGGGTTA CCCAAAGTTC TCTCAGGGGG ACCCCATGAG  
 30701 TCTTTGCGCC TATCCGAACC TCTAGTTACC TCCAATGGCA TGCTTGCGCT  
 AGAAACGCGG ATAGGCTTGG AGATCAATGG AGGTTACCGT ACGAACGCGA  
 30751 CAAAATGGGC AACGGCCTCT CTCTGGACGA GGCCGGCAAC CTTACCTCCC  
 GTTTTACCCG TTGCCGGAGA GAGACCTGCT CCGGCCGTTG GAATGGAGGG  
 30801 AAAATGTAAC CACTGTGAGC CCACCTCTCA AAAAAACCAA GTCAAACATA  
 TTTTACATTG GTGACACTCG GGTGGAGAGT TTTTTTGGTT CAGTTTGTAT  
 30851 AACCTGGAAA TATCTGCACC CCTCACAGTT ACCTCAGAAG CCCTAACTGT  
 TTGGACCTTT ATAGACGTGG GGAGTGTCAA TGGAGTCTTC GGGATTGACA  
 30901 GGCTGCCGCC GCACCTCTAA TGGTCGCGGG CAACACACTC ACCATGCAAT  
 CCGACGGCGG CGTGGAGATT ACCAGCGCCC GTTGTGTGAG TGGTACGTTA  
 30951 CACAGGCCCC GCTAACCGTG CACGACTCCA AACTTAGCAT TGCCACCCAA  
 GTGTCCGGGG CGATTGGCAC GTGCTGAGGT TTGAATCGTA ACGGTGGGTT  
 31001 GGACCCCTCA CAGTGTGAGA AGGAAAGCTA GCCCTGCAA CATCAGGCCC  
 CCTGGGGAGT GTCACAGTCT TCCTTTCGAT CGGGACGTTT GTAGTCCGGG  
 31051 CCTCACCACC ACCGATAGCA GTACCCTTAC TATCACTGCC TCACCCCTT  
 GGAGTGGTGG TGGCTATCGT CATGGGAATG ATAGTGACGG AGTGGGGGAA  
 31101 TAACTACTGC CACTGGTAGC TTGGGCATTG ACTTGAAAGA GCCCATTAT  
 ATTGATGACG GTGACCATCG AACCCTGAAC TGAACCTTCT CGGGTAAATA  
 31151 ACACAAAATG GAAAACTAGG ACTAAAGTAC GGGGCTCCTT TGCATGTAAC  
 TGTGTTTTAC CTTTTGATCC TGATTTCATG CCCCAGGAA ACGTACATTG  
 31201 AGACGACCTA AACACTTTGA CCGTAGCAAC TGGTCCAGGT GTGACTATTA  
 TCTGCTGGAT TTGTGAAACT GGCATCGTTG ACCAGGTCCA CACTGATAAT  
 31251 ATAATACTTC CTTGCAAACCT AAAGTTACTG GAGCCTTGGG TTTTGATTCA  
 TATTATGAAG GAACGTTTGA TTTCAATGAC CTCGGAACCC AAAACTAAGT  
 31301 CAAGGCAATA TGCAACTTAA TGTAGCAGGA GGACTAAGGA TTGATTCTCA  
 GTTCCGTTAT ACGTTGAATT ACATCGTCCT CCTGATTCTT AACTAAGAGT  
 31351 AAACAGACGC CTTATACTTG ATGTTAGTTA TCCGTTTGAT GCTCAAAACC  
 TTTGTCTGCG GAATATGAAC TACAATCAAT AGGCAAACCTA CGAGTTTTGG

FIG.9A-37

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31401	AACTAAATCT	AAGACTAGGA	CAGGGCCCTC	TTTTTATAAA	CTCAGCCCAC
	TTGATTTAGA	TTCTGATCCT	GTCCC GGGAG	AAAAATATTT	GAGTCGGGTG
31451	AACTTGGATA	TTAACTACAA	CAAAGGCCTT	TACTTGTTTA	CAGCTTCAAA
	TTGAACCTAT	AATTGATGTT	GTTTCCGGAA	ATGAACAAAT	GTCGAAGTTT
31501	CAATTCCAAA	AAGCTTGAGG	TTAACCTAAG	CACTGCCAAG	GGGTTGATGT
	GTTAAGGTTT	TTCGAACCTC	AATTGGATTG	GTGACGGTTC	CCCAACTACA
31551	TTGACGCTAC	AGCCATAGCC	ATTAATGCAG	GAGATGGGCT	TGAATTTGGT
	AACTGCGATG	TCGGTATCGG	TAATTACGTC	CTCTACCCGA	ACTTAAACCA
31601	TCACCTAATG	CACCAAACAC	AAATCCCCTC	AAAACAAAAA	TTGGCCATGG
	AGTGGATTAC	GTGGTTTGTG	TTAGGGGAG	TTTTGTTTTT	AACCGGTACC
31651	CCTAGAATTT	GATTCAAACA	AGGCTATGGT	TCCTAAACTA	GGAAGTGGCC
	GGATCTTAAA	CTAAGTTTGT	TCCGATACCA	AGGATTTGAT	CCTTGACCGG
31701	TTAGTTTTGA	CAGCACAGGT	GCCATTACAG	TAGGAAACAA	AAATAATGAT
	AATCAAAACT	GTCGTGTCCA	CGGTAATGTC	ATCCTTTGTT	TTTATTACTA
31751	AAGCTAACTT	TGTGGACCAC	ACCAGCTCCA	TCTCCTAACT	GTAGACTAAA
	TTCGATTGAA	ACACCTGGTG	TGGTCGAGGT	AGAGGATTGA	CATCTGATTT
31801	TGCAGAGAAA	GATGCTAAAC	TCACTTTGGT	CTTAACAAAA	TGTGGCAGTC
	ACGTCTCTTT	CTACGATTTG	AGTGAAACCA	GAATTGTTTT	ACACCGTCAG
31851	AAATACTTGC	TACAGTTTCA	GTTTTGGCTG	TTAAAGGCAG	TTTGGCTCCA
	TTTATGAACG	ATGTCAAAGT	CAAAACCGAC	AATTTCCGTC	AAACCGAGGT
31901	ATATCTGGAA	CAGTTCAAAG	TGCTCATCTT	ATTATAAGAT	TTGACGAAAA
	TATAGACCTT	GTCAAGTTTC	ACGAGTAGAA	TAATATTCTA	AACTGCTTTT
31951	TGGAGTGCTA	CTAAACAATT	CCTTCCTGGA	CCCAGAATAT	TGGAACTTTA
	ACCTCACGAT	GATTTGTTAA	GGAAGGACCT	GGGTCTTATA	ACCTTGAAAT
32001	GAAATGGAGA	TCTTACTGAA	GGCACAGCCT	ATACAAACGC	TGTTGGATTT
	CTTTACCTCT	AGAATGACTT	CCGTGTCGGA	TATGTTTGCG	ACAACCTAAA
32051	ATGCCTAACC	TATCAGCTTA	TCCAAAATCT	CACGGTAAAA	CTGCCAAAAG
	TACGGATTGG	ATAGTCGAAT	AGGTTTTAGA	GTGCCATTTT	GACGGTTTTT
32101	TAACATTGTC	AGTCAAGTTT	ACTTAAACGG	AGACAAAAC	AAACCTGTAA
	ATTGTAACAG	TCAGTTCAAA	TGAATTTGCC	TCTGTTTTGA	TTTGGACATT
32151	CACTAACCAT	TACACTAAAC	GGTACACAGG	AAACAGGAGA	CACAACTCCA
	GTGATTGGTA	ATGTGATTTG	CCATGTGTCC	TTTGTCTCT	GTGTTGAGGT
32201	AGTGCATACT	CTATGTCATT	TTCATGGGAC	TGGTCTGGCC	ACAACTACAT
	TCACGTATGA	GATACAGTAA	AAGTACCCTG	ACCAGACCGG	TGTTGATGTA

FIG.9A-38

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32251	TAATGAAATA ATTACTTTAT	TTTGCCACAT AAACGGTGTA	CCTCTTACAC GGAGAATGTG	TTTTTCATAC AAAAAGTATG	ATTGCCCAAG TAACGGGTTC
32301	AATAAAGAAT TTATTTCTTA	CGTTTGTGTT GCAAACACAA	ATGTTTCAAC TACAAAGTTG	GTGTTTATTT CACAAATAAA	TTCAATTGCA AAGTTAACGT
32351	GAAAATTTCA CTTTTAAAGT	AGTCATTTTT TCAGTAAAAA	CATTCAAGTAG GTAAGTCATC	TATAGCCCCA ATATCGGGGT	CCACCACATA GGTGGTGTAT
32401	GCTTATACAG CGAATATGTC	ATCACCGTAC TAGTGGCATG	CTTAATCAAA GAATTAGTTT	CTCACAGAAC GAGTGTCTTG	CCTAGTATTC GGATCATAAG
32451	AACCTGCCAC TTGGACGGTG	CTCCCTCCCA GAGGGAGGGT	ACACACAGAG TGTGTGTCTC	TACACAGTCC ATGTGTCAGG	TTTCTCCCCG AAAGAGGGGC
32501	GCTGGCCTTA CGACCGGAAT	AAAAGCATCA TTTTCGTAGT	TATCATGGGT ATAGTACCCA	AACAGACATA TTGTCTGTAT	TTCTTAGGTG AAGAATCCAC
32551	TTATATTCCA AATATAAGGT	CACGGTTTTCC GTGCCAAAGG	TGTCGAGCCA ACAGCTCGGT	AACGCTCATC TTGCGAGTAG	AGTGATATTA TCACTATAAT
32601	ATAAACTCCC TATTTGAGGG	CGGGCAGCTC GCCCCTCGAG	ACTTAAGTTC TGAATTCAAG	ATGTCGCTGT TACAGCGACA	CCAGCTGCTG GGTCGACGAC
32651	AGCCACAGGC TCGGTGTCCG	TGCTGTCCAA ACGACAGGTT	CTTGCGGTTG GAACGCCAAC	CTTAACGGGC GAATTGCCCC	GGCGAAGGAG CCGCTTCCTC
32701	AAGTCCACGC TTCAGGTGCG	CTACATGGGG GATGTACCCC	GTAGAGTCAT CATCTCAGTA	AATCGTGCAT TTAGCACGTA	CAGGATAGGG GTCCTATCCC
32751	CGGTGGTGCT GCCACCACGA	GCAGCAGCGC CGTCGTGCGG	GCGAATAAAC CGCTTATTTG	TGCTGCCGCC ACGACGGCGG	GCCGCTCCGT CGGCGAGGCA
32801	CCTGCAGGAA GGACGTCCTT	TACAACATGG ATGTTGTACC	CAGTGGTCTC GTCACCAGAG	CTCAGCGATG GAGTCGCTAC	ATTCGCACCG TAAGCGTGCG
32851	CCCGCAGCAT GGGCGTCGTA	AAGGCGCCTT TTCCGCGGAA	GTCCTCCGGG CAGGAGGCC	CACAGCAGCG GTGTCGTGCG	CACCCTGATC GTGGGACTAG
32901	TCACTTAAAT AGTGAATTTA	CAGCACAGTA GTCGTGTCAT	ACTGCAGCAC TGACGTCGTG	AGCACCACAA TCGTGGTGTT	TATTGTTCAA ATAACAAGTT
32951	AATCCACAG TTAGGGTGTC	TGCAAGGCGC ACGTTCCGCG	TGTATCCAAA ACATAGGTTT	GTCATGGCG CGAGTACCGC	GGGACCACAG CCCTGGTGTC
33001	AACCCACGTG TTGGGTGCAC	GCCATCATAC CGGTAGTATG	CACAAGCGCA GTGTTGCGGT	GGTAGATTAA CCATCTAATT	GTGGCGACCC CACCCTGGG
33051	CTCATAAACA GAGTATTTGT	CGCTGGACAT GCGACCTGTA	AAACATTACC TTTGTAATGG	TCTTTTGGCA AGAAAACCGT	TGTTGTAATT ACAACATTAA

FIG.9A-39

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33101	CACCACCTCC GTGGTGGAGG	CGGTACCATA GCCATGGTAT	TAAACCTCTG ATTTGGAGAC	ATTAAACATG TAATTTGTAC	GCGCCATCCA CGCGGTAGGT
33151	CCACCATCCT GGTGGTAGGA	AAACCAGCTG TTTGGTCGAC	GCCAAAACCT CGGTTTTGGA	GCCCGCCGGC CGGGCGGCCG	TATACACTGC ATATGTGACG
33201	AGGGAACCGG TCCCTTGGCC	GA CTGGAACA CTGACCTTGT	ATGACAGTGG TACTGTCACC	AGAGCCCAGG TCTCGGGTCC	ACTCGTAACC TGAGCATTGG
33251	ATGGATCATC TACCTAGTAG	ATGCTCGTCA TACGAGCAGT	TGATATCAAT ACTATAGTTA	GTTGGCACAA CAACCGTGTT	CACAGGCACA GTGTCCGTGT
33301	CGTGCATACA GCACGTATGT	CTTCCTCAGG GAAGGAGTCC	ATTACAAGCT TAATGTTCTGA	CCTCCCGCGT GGAGGGCGCA	TAGAACCATA ATCTTGGTAT
33351	TCCCAGGGAA AGGGTCCCTT	CAACCCATTG GTTGGGTAAG	CTGAATCAGC GACTTAGTCTG	GTAAATCCCA CATTTAGGGT	CACTGCAGGG GTGACGTCCC
33401	AAGACCTCGC TTCTGGAGCG	ACGTA ACTCA TGCATTGAGT	CGTTGTGCAT GCAACACGTA	TGTCAAAGTG ACAGTTTCAC	TTACATTTCGG AATGTAAGCC
33451	GCAGCAGCGG CGTCGTCGCC	ATGATCCTCC TACTAGGAGG	AGTATGGTAG TCATACCATC	CGCGGGTTTC GCGCCCAAAG	TGTCTCAAAA ACAGAGTTTT
33501	GGAGGTAGAC CCTCCATCTG	GATCCCTACT CTAGGGATGA	GTACGGAGTG CATGCCTCAC	CGCCGAGACA GCGGCTCTGT	ACCGAGATCG TGGCTCTAGC
33551	TGTTGGTCGT ACAACCAGCA	AGTGT CATGC TCACAGTACG	CAAATGGAAC GTTTACCTTG	GCCGGACGTA CGGCCTGCAT	GTCATATTTTC CAGTATAAAG
33601	CTGAAGCAAA GACTTCGTTT	ACCAGGTGCG TGGTCCACGC	GGCGTGACAA CCGCACTGTT	ACAGATCTGC TGTCTAGACG	GTCTCCGGTC CAGAGGCCAG
33651	TCGCCGCTTA AGCGGCGAAT	GATCGCTCTG CTAGCGAGAC	TGTAGTAGTT ACATCATCAA	G TAGTATATC CATCATATAG	CACTCTCTCA GTGAGAGAGT
33701	AAGCATCCAG TTCGTAGGTC	GCGCCCCCTG CGCGGGGGAC	GCTTCGGGTT CGAAGCCCAA	CTATGTAAAC GATACATTTG	TCCTTCATGC AGGAAGTACG
33751	GCCGCTGCCC CGGCGACGGG	TGATAACATC ACTATTGTAG	CACCACCGCA GTGGTGGCGT	GAATAAGCCA CTTATTCGGT	CACCCAGCCA GTGGGTGCGT
33801	ACCTACACAT TGGATGTGTA	TCGTTCTGCG AGCAAGACGC	AGTCACACAC TCAGTGTGTG	GGGAGGAGCG CCCTCCTCGC	GGAAGAGCTG CCTTCTCGAC
33851	GAAGAACCAT CTTCTTGGTA	GTTTTTTTTT CAAAAAAAAAA	TTATTCCAAA AATAAGGTTT	AGATTATCCA TCTAATAGGT	AAACCTCAAA TTTGGAGTTT
33901	ATGAAGATCT TACTTCTAGA	ATTAAGTGAA TAATTCACCT	CGCGCTCCCC GCGCGAGGGG	TCCGGTGGCG AGGCCACCGC	TGGTCAAACCT ACCAAGTTTGA

FIG.9A-40

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33951	CTACAGCCAA	AGAACAGATA	ATGGCATTG	TAAGATGTTG	CACAATGGCT
	GATGTCGGTT	TCTTGTCTAT	TACCGTAAAC	ATTCTACAAC	GTGTTACCGA
34001	TCCAAAAGGC	AAACGGCCCT	CACGTCCAAG	TGGACGTAAA	GGCTAAACCC
	AGGTTTTCCG	TTTGCCGGGA	GTGCAGGTTT	ACCTGCATT	CCGATTGGG
34051	TTCAGGGTGA	ATCTCCTCTA	TAAACATTCC	AGCACCTTCA	ACCATGCCCA
	AAGTCCCACT	TAGAGGAGAT	ATTTGTAAGG	TCGTGGAAGT	TGGTACGGGT
34101	AATAATTCTC	ATCTCGCCAC	CTTCTCAATA	TATCTCTAAG	CAAATCCCGA
	TTATTAAGAG	TAGAGCGGTG	GAAGAGTTAT	ATAGAGATT	GTTTAGGGCT
34151	ATATTAAGTC	CGGCCATTGT	AAAAATCTGC	TCCAGAGCGC	CCTCCACCTT
	TATAATTGAG	GCCGGTAACA	TTTTTAGACG	AGGTCTCGCG	GGAGGTGGAA
34201	CAGCCTCAAG	CAGCGAATCA	TGATTGCAAA	AATTCAGGTT	CCTCACAGAC
	GTCGGAGTTC	GTCGCTTAGT	ACTAACGTTT	TTAAGTCCAA	GGAGTGTCTG
34251	CTGTATAAGA	TTCAAAAGCG	GAACATTAAC	AAAAATACCG	CGATCCCGTA
	GACATATTCT	AAGTTTTTCG	CTTGTAATTG	TTTTTATGGC	GCTAGGGCAT
34301	GGTCCCTTCG	CAGGGCCAGC	TGAACATAAT	CGTGCAGGTC	TGCACGGACC
	CCAGGGGAAGC	GTCCCGGTG	ACTTGTATTA	GCACGTCCAG	ACGTGCCTGG
34351	AGCGCGGCCA	CTTCCCCGCC	AGGAACCATG	ACAAAAGAAC	CCCACTGAT
	TCGCGCCGGT	GAAGGGGCGG	TCCTTGGTAC	TGTTTTCTTG	GGTGTGACTA
34401	TATGACACGC	ATACTCGGAG	CTATGCTAAC	CAGCGTAGCC	CCGATGTAAG
	ATACTGTGCG	TATGAGCCTC	GATACGATTG	GTCGCATCGG	GGCTACATTC
34451	CTTGTTGCAT	GGGCGGCGAT	ATAAAATGCA	AGGTGCTGCT	CAAAAAATCA
	GAACAACGTA	CCCGCCGCTA	TATTTTACGT	TCCACGACGA	GTTTTTTAGT
34501	GGCAAAGCCT	CGCGCAAAAA	AGAAAGCACA	TCGTAGTCAT	GCTCATGCAG
	CCGTTTCGGA	GCGCGTTTTT	TCTTTCGTGT	AGCATCAGTA	CGAGTACGTC
34551	ATAAAGGCAG	GTAAGCTCCG	GAACCACCAC	AGAAAAAGAC	ACCATTTTTTC
	TATTTCCGTC	CATTCGAGGC	CTTGGTGGTG	TCTTTTTCTG	TGGTAAAAAG
34601	TCTCAAACAT	GTCTGCGGGT	TTCTGCATAA	ACACAAAATA	AAATAACAAA
	AGAGTTTGTA	CAGACGCCCA	AAGACGTATT	TGTGTTTTAT	TTTATTGTTT
34651	AAAACATTTA	AACATTAGAA	GCCTGTCTTA	CAACAGGAAA	AACAACCTT
	TTTTGTAAAT	TTGTAATCTT	CGGACAGAAT	GTTGTCCTTT	TTGTTGGGAA
34701	ATAAGCATAA	GACGGACTAC	GGCCATGCCG	GCGTGACCGT	AAAAAACTG
	TATTCGTATT	CTGCCTGATG	CCGGTACGGC	CGCACTGGCA	TTTTTTTGAC
34751	GTCACCGTGA	TTAAAAAGCA	CCACCGACAG	CTCCTCGGTC	ATGTCCGGAG
	CAGTGGCACT	AATTTTTTCG	GGTGGCTGTC	GAGGAGCCAG	TACAGGCCTC

FIG.9A-41

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34801 TCATAATGTA AGACTCGGTA AACACATCAG GTTGATTCAC ATCGGTCAGT  
 AGTATTACAT TCTGAGCCAT TTGTGTAGTC CAACTAAGTG TAGCCAGTCA  
 34851 GCTAAAAAGC GACCGAAATA GCCCGGGGGA ATACATACCC GCAGGCGTAG  
 CGATTTTTCG CTGGCTTTAT CGGGCCCCCT TATGTATGGG CGTCCGCATC  
 34901 AGACAACATT ACAGCCCCCA TAGGAGGTAT AACAAAATTA ATAGGAGAGA  
 TCTGTTGTAA TGTCGGGGGT ATCCTCCATA TTGTTTTAAT TATCCTCTCT  
 34951 AAAACACATA AACACCTGAA AAACCCTCCT GCCTAGGCAA AATAGCACCC  
 TTTTGTGTAT TTGTGGACTT TTTGGGAGGA CGGATCCGTT TTATCGTGGG  
 35001 TCCCGCTCCA GAACAACATA CAGCGCTTCC ACAGCGGCAG CCATAACAGT  
 AGGGCGAGGT CTTGTTGTAT GTCGCGAAGG TGTCGCCGTC GGTATTGTCA  
 35051 CAGCCTTACC AGTAAAAAG AAAACCTATT AAAAAACAC CACTCGACAC  
 GTCGGAATGG TCATTTTTTC TTTTGGATAA TTTTTTTGTG GTGAGCTGTG  
 35101 GGCACCAGCT CAATCAGTCA CAGTGTAATA AAGGGCCAAG TGCAGAGCGA  
 CCGTGGTCGA GTTAGTCAGT GTCACATTTT TTCCCGGTTT ACGTCTCGCT  
 35151 GTATATATAG GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA  
 CATATATATC CTGATTTTTT ACTGCATTGC CAATTTTCAGG TGTTTTTTGT  
 35201 CCCAGAAAAC CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC  
 GGGTCTTTTG GCGTGCGCTT GGATGCGGGT CTTTGCTTTC GGTTTTTTGG  
 35251 CACAACCTTCC TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTCACTTCC  
 GTGTTGAAGG AGTTTAGCAG TGAAGGCAAA AGGGTGCAAT GCAGTGAAGG  
 35301 CATTTTAAGA AAACCTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA  
 GTAAAATTCT TTTGATGTTA AGGGTTGTGT ATGTTCAATG AGGCGGGATT  
 35351 AACCTACGTC ACCCGCCCCG TTCCACGACC CCGCGCCACG TCACAAACTC  
 TTGGATGCAG TGGCGGGGGC AAGGGTGCGG GGCAGCGTGC AGTGTGTTGAG  
 35401 CACCCCTCA TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG  
 GTGGGGGAGT AATAGTATAA CCGAAGTTAG GTTTTATTCC ATATAATAAC

PacI

35451 ATGATGTTAA TTAAGAATTC GGATCTGCGA CGCGAGGCTG GATGGCCTTC  
 TACTACAATT AATTCTTAAG CCTAGACGCT GCGCTCCGAC CTACCGGAAG  
 35501 CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA  
 GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCAGCAACGT  
 35551 GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG  
 CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC

FIG.9A-42

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35601	GCCAGCAAAA CGGTCGTTTT	GGCCAGGAAC CCGGTCCTTG	CGTAAAAAGG GCATTTTTTC	CCGCGTTGCT GGCGCAACGA	GGCGTTTTTC CCGCAAAAAG
35651	CATAGGCTCC GTATCCGAGG	GGCCCCCTGA CGGGGGGACT	CGAGCATCAC GCTCGTAGTG	AAAAATCGAC TTTTTAGCTG	GCTCAAGTCA CGAGTTCAGT
35701	GAGGTGGCGA CTCCACCGCT	AACCCGACAG TTGGGCTGTC	GACTATAAAG CTGATATTTT	ATACCAGGCG TATGGTCCGC	TTTCCCCCTG AAAGGGGGAC
35751	GAAGCTCCCT CTTCGAGGGA	CGTGCGCTCT GCACGCGAGA	CCTGTTCCGA GGACAAGGCT	CCCTGCCGCT GGGACGGCGA	TACCGGATAC ATGGCCTATG
35801	CTGTCCGCCT GACAGGCGGA	TTCTCCCTTC AAGAGGGAAG	GGGAAGCGTG CCCTTCGCAC	GCGCTTTTCT CGCGAAAGAG	ATAGCTCACG TATCGAGTGC
35851	CTGTAGGTAT GACATCCATA	CTCAGTTCGG GAGTCAAGCC	TGTAGGTCGT ACATCCAGCA	TCGCTCCAAG AGCGAGGTTC	CTGGGCTGTG GACCCGACAC
35901	TGCACGAACC ACGTGCTTGG	CCCCGTTTCA GGGGCAAGTC	CCCGACCGCT GGGCTGGCGA	GCGCCTTATC CGCGGAATAG	CGGTAACAT GCCATTGATA
35951	CGTCTTGAGT GCAGAACTCA	CCAACCCGGT GGTTGGGCCA	AAGACACGAC TTCTGTGCTG	TTATCGCCAC AATAGCGGTG	TGGCAGCAGC ACCGTCGTCG
36001	CACTGGTAAC GTGACCATTG	AGGATTAGCA TCCTAATCGT	GAGCGAGGTA CTCGCTCCAT	TGTAGGCGGT ACATCCGCCA	GCTACAGAGT CGATGTCTCA
36051	TCTTGAAGTG AGAACTTCAC	GTGGCCTAAC CACCGGATTG	TACGGCTACA ATGCCGATGT	CTAGAAGGAC GATCTTCCTG	AGTATTTGGT TCATAAACCA
36101	ATCTGCGCTC TAGACGCGAG	TGCTGAAGCC ACGACTTCGG	AGTTACCTTC TCAATGGAAG	GGAAAAAGAG CCTTTTTTCT	TTGGTAGCTC AACCATCGAG
36151	TTGATCCGGC AACTAGGCCG	AAACAAACCA TTTGTTTGGT	CCGCTGGTAG GGCGACCATC	CGGTGGTTTT GCCACCAAAA	TTTGTTTGCA AAACAAACGT
36201	AGCAGCAGAT TCGTGCTCTA	TACGCGCAGA ATGCGCGTCT	AAAAAAGGAT TTTTTTCCTA	CTCAAGAAGA GAGTTCTTCT	TCCTTTGATC AGGAAACTAG
36251	TTTTCTACGG AAAAGATGCC	GGTCTGACGC CCAGACTGCG	TCAGTGGAAC AGTCACCTTG	GAAAACTCAC CTTTTGAGTG	GTTAAGGGAT CAATTCCCTA
36301	TTTGGTCATG AAACCAGTAC	AGATTATCAA TCTAATAGTT	AAAGGATCTT TTTCCTAGAA	CACCTAGATC GTGGATCTAG	CTTTTAAATC GAAAATTTAG
36351	AATCTAAAGT TTAGATTTCA	ATATATGAGT TATATACTCA	AAACTTGGTC TTTGAACCAG	TGACAGTTAC ACTGTCAATG	CAATGCTTAA GTTACGAATT
36401	TCAGTGAGGC AGTCACTCCG	ACCTATCTCA TGGATAGAGT	GCGATCTGTC CGCTAGACAG	TATTTTCGTT ATAAAGCAAG	ATCCATAGTT TAGGTATCAA

FIG.9A-43

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36451	GCCTGACTCC CGGACTGAGG	CCGTCGTGTA GGCAGCACAT	GATAACTACG CTATTGATGC	ATACGGGAGG TATGCCCTCC	GCTTACCATC CGAATGGTAG
36501	TGGCCCCAGT ACCGGGGTCA	GCTGCAATGA CGACGTTACT	TACCGCGAGA ATGGCGCTCT	CCCACGCTCA GGGTGCGAGT	CCGGCTCCAG GGCCGAGGTC
36551	ATTTATCAGC TAAATAGTCG	AATAAACCCAG TTATTTGGTC	CCAGCCGGAA GGTCGGCCTT	GGGCCGAGCG CCCGGCTCGC	CAGAAGTGGT GTCTTCACCA
36601	CCTGCAACTT GGACGTTGAA	TATCCGCCTC ATAGGCGGAG	CATCCAGTCT GTAGGTCAGA	ATTAATTGTT TAATTAACAA	GCCGGGAAGC CGGCCCTTCG
36651	TAGAGTAAGT ATCTCATTCA	AGTTCGCCAG TCAAGCGGTC	TTAATAGTTT AATTATCAAA	GCGCAACGTT CGCGTTGCAA	GTTGCCATTG CAACGGTAAC
36701	CTACAGGCAT GATGTCCGTA	CGTGGTGTCA GCACCACAGT	CGCTCGTCGT GCGAGCAGCA	TTGGTATGGC AACCATACCG	TTCATTACAGC AAGTAAGTCG
36751	TCCGGTTCCC AGGCCAAGGG	AACGATCAAG TTGCTAGTTC	GCGAGTTACA CGCTCAATGT	TGATCCCCCA ACTAGGGGGT	TGTTGTGCAA ACAACACGTT
36801	AAAAGCGGTT TTTTCGCCAA	AGCTCCTTCG TCGAGGAAGC	GTCCTCCGAT CAGGAGGCTA	CGTTGTCAGA GCAACAGTCT	AGTAAGTTGG TCATTCAACC
36851	CCGCAGTGTT GGCGTCACAA	ATCACTCATG TAGTGAGTAC	GTTATGGCAG CAATACCGTC	CACTGCATAA GTGACGTATT	TTCTCTTACT AAGAGAATGA
36901	GTCATGCCAT CAGTACGGTA	CCGTAAGATG GGCATTCTAC	CTTTTCTGTG GAAAAGACAC	ACTGGTGAGT TGACCACTCA	ACTCAACCAA TGAGTTGGTT
36951	GTCATTCTGA CAGTAAGACT	GAATAGTGTA CTTATCACAT	TGCGGCGACC ACGCCGCTGG	GAGTTGCTCT CTCAACGAGA	TGCCCCGGCGT ACGGGCCGCA
37001	CAACACGGGA GTTGTGCCCT	TAATACCGCG ATTATGGCGC	CCACATAGCA GGTGTATCGT	GAACTTTAAA CTTGAAATTT	AGTGCTCATC TCACGAGTAG
37051	ATTGGAAAAC TAACCTTTTG	GTTCTTCGGG CAAGAAGCCC	GCGAAAACCTC CGCTTTTGAG	TCAAGGATCT AGTTCCTAGA	TACCGCTGTT ATGGCGACAA
37101	GAGATCCAGT CTCTAGGTCA	TCGATGTAAC AGCTACATTG	CCACTCGTGC GGTGAGCAGC	ACCCAACCTGA TGGGTTGACT	TCTTCAGCAT AGAAGTCGTA
37151	CTTTTACTTT GAAAATGAAA	CACCAGCGTT GTGGTCGCAA	TCTGGGTGAG AGACCCACTC	CAAAAACAGG GTTTTTGTCC	AAGGCAAAAT TTCCGTTTTA
37201	GCCGCAAAAA CGGCGTTTTT	AGGGAATAAG TCCCTTATTC	GGCGACACGG CCGCTGTGCC	AAATGTTGAA TTTACAACCT	TACTCATACT ATGAGTATGA
37251	CTTCCTTTTT GAAGGAAAAA	CAATATTATT GTTATAATAA	GAAGCATTTA CTTCGTAAAT	TCAGGGTTAT AGTCCCAATA	TGTCTCATGA ACAGAGTACT

FIG.9A-44



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37301 GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG  
CGCCTATGTA TAAACTTACA TAAATCTTTT TATTTGTTTA TCCCAAGGC

37351 CGCACATTTT CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT  
GCGTGTAAG GGGCTTTTCA CGGTGGACTG CAGATTCTTT GGTAATAATA

37401 CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCC TTTTCGTCTTC  
GTACTGTAAT TGGATATTTT TATCCGCATA GTGCTCCGGG AAAGCAGAAG

37451 AAGAATTGGA TCCGAATTCT TAAT  
TTCTTAACCT AGGCTTAAGA ATTA

FIG.9A-45

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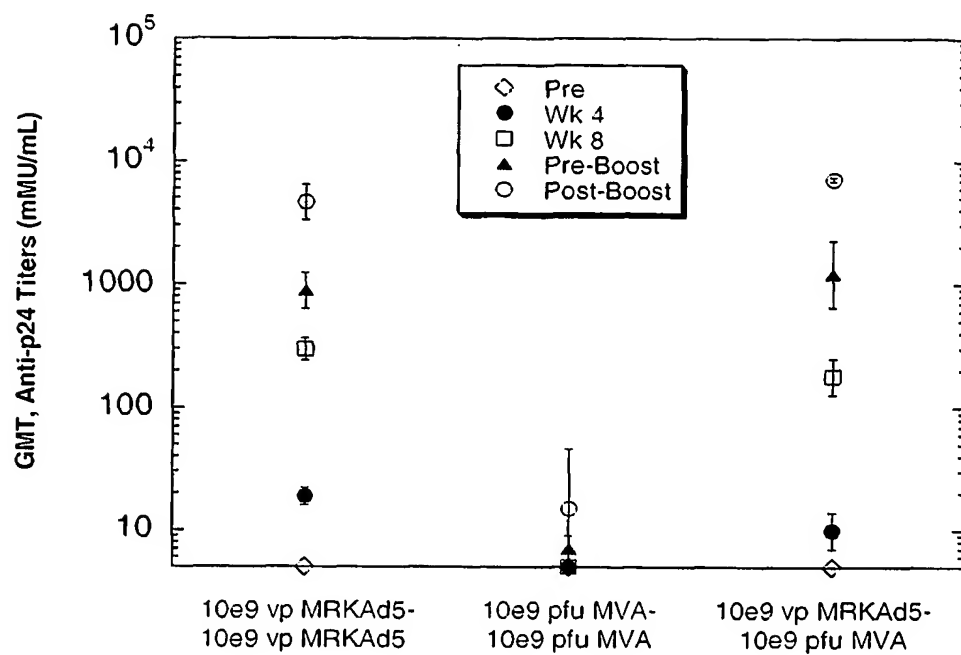


FIG. 10

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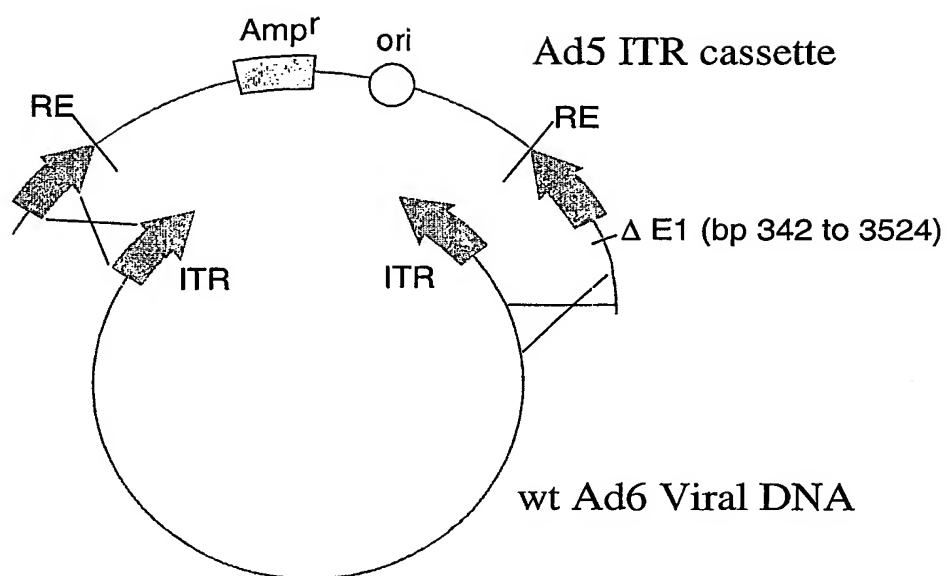


FIG. 11

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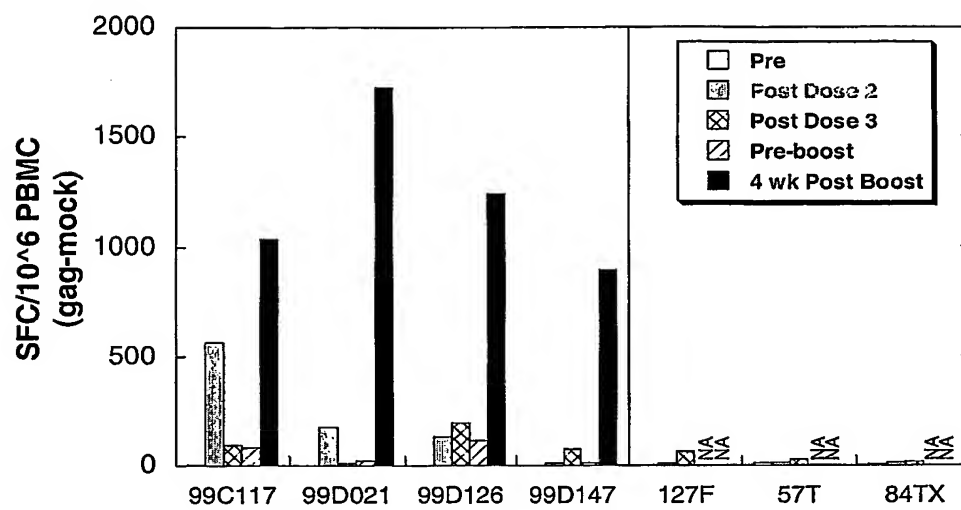


FIG. 12